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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster / discussion, workshops, roundtables, and poster sessions of the 35th Annual Meeting of the Society of Toxicology, held at the Anaheim Convention Center, Anaheim, California, March 10-14, 1996.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 351.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 375.

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

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1 REPAIR OF DNA DAMAGE: MECHANISMS AND CONSEQUENCES

W M Baird[1], P C Hanawalt[2], V M Maher[3]. [1]*Purdue University, West Lafayette, IN,* [2]*Department of Biological Sciences, Stanford University, Stanford, CA,* [3]*Carcinogenesis Laboratory, The Cancer Center, Michigan State University, East Lansing, MI*

Numerous chemicals exert toxic effects through their interactions with DNA. These genotoxic agents range from drugs used in chemotherapy to many classes of environmental carcinogens such as alkylating agents, combustion products, compounds from plants and dye intermediates. These interactions can result in the induction of mutations that can lead to cancer teratogenesis and a number of other deleterious effects. A major determinant of whether such toxicological consequences occur is the ability of the individual to repair this damage to DNA prior to DNA replication and the fixation of a mutation. To understand the consequences of exposure to genotoxic compounds, it is essential to understand how DNA damage is repaired. It is now recognized that DNA repair is not uniform throughout the genome but occurs selectively in certain areas. This selection is dependent upon the type of DNA damage induced as well as the area of the genome. The symposium is designed to provide an introduction to repair in the genome overall and to describe repair within specific genes and DNA strands and its relationship to transcriptional activity. The recent development of approaches for examining repair at specific sites in the DNA of the genome using ligation-mediated PCR will be described. Overall, this symposium will provide a thorough, contemporary overview of DNA repair and its role in the response to exposure to genotoxic agents.

2 REPAIR OF DNA DAMAGE IN THE TOTAL GENOME AS MEASURED BY ANALYSIS OF DNA ADDUCTS

W M Baird. *Department of Medicinal Chemistry, Purdue University, West Lafayette, IN*

The repair of DNA damage from many types of genotoxic agents was initially measured by determining the amount of adducts present in the DNA of the cell or tissue at various lengths of time after exposure. The level of DNA adducts present in cells treated with doses of genotoxic agents that allowed cell survival, frequently in the range of one adduct per 10^5 nucleotides or less, necessitated the development of sensitive analytical techniques. Many adducts were detected by treating the cells with radioisotope-labeled genotoxic compounds, but such studies were restricted to those compounds for which suitable levels of radioisotope labeling could be achieved. The development of ^{32}P postlabeling by Randerath, Gupta and coworkers allowed measurement of DNA adducts of a wide range of genotoxic agents from drugs used in chemotherapy to environmental carcinogens such as polycyclic aromatic hydrocarbons. This presentation will discuss recent advances in postlabeling analytical procedures for the measurement of DNA adducts, especially those of polycyclic aromatic hydrocarbons, including the use of isotopes such as ^{33}P and ^{35}S and procedures for adduct separation by HPLC. The use of these procedures to measure the repair of specific carcinogen adducts from the total genome will be described. The relationship of repair of specific DNA adducts to the induction of mutations and carcinogenesis will be discussed. Supported by grants CA 40228 and CA28825. NCI, DHHS.

3 REPAIR OF DNA DAMAGE IN TRANSCRIPTIONALLY ACTIVE GENES

P C Hanawalt. *Department of Biological Sciences, Stanford University, Stanford, CA*

Repair of DNA damage is not uniform throughout the genome. Studies of the removal of cyclobutane pyrimidine dimers from the genome of mammalian cells in culture have demonstrated that they are repaired at a greater rate in transcriptionally active genes than in nontranscribed regions. This, so called, transcription-coupled repair results in the selective removal of damage from the DNA strand being transcribed within transcription units, as has been documented in bacteria, yeast, and mammalian cells. There is a consequent increase in the proportion of mutations that result from miscoding due to persisting DNA lesions in the nontranscribed strand. Transcription-coupled repair removes DNA lesions resulting from both physical and chemical carcinogens including UV light, cisplatin, nitrogen mustard, and some polycyclic aromatic hydrocarbon diol epoxides. This presentation will discuss the excision-repair mechanism, techniques used to measure lesions and their repair in specific genes (and specific strands of DNA), models for transcription-coupled repair and the types of DNA damage repaired by this mechanism. Repair studies on cells from victims of the cancer-prone hereditary diseases, xeroderma pigmentosum and Li-Fraumeni syndrome, will be described as examples of how the survival of damaged cells does not necessarily correlate with global genomic repair efficiencies - but it may correlate with transcription-coupled repair. The biological consequences of transcription-coupled repair and their relationships to toxicological endpoints such as mutation and carcinogenesis will be discussed. Supported by an outstanding investigator grant (CA 44349) from the National Cancer Institute.

4 STRAND-SPECIFIC AND SITE SPECIFIC REPAIR OF BPDE ADDUCTS IN THE *HPRT* GENE OF HUMAN CELLS: CORRELATION WITH MUTATION SPECTRA

V M Maher, R H Chen, D Wei, and J J McCormick. *Carcinogenesis Laboratory, The Cancer Center, Michigan State University, East Lansing, MI*

BPDE binds to the N^2 position of guanine. To investigate the effect of repair on the spectrum of mutations induced in human cells by BPDE, we synchronized and exposed them in early S-phase or 12 h before S (early G_1) to allow ~ 12 h for repair before DNA replication. The frequency of *HPRT* mutants was 300 X 10^{-6} for cells treated in early S and 100 X 10^{-6} for cells with time for repair. For cells treated in S, 24% of the lesions responsible for the mutations were located in the transcribed strand; for G_1-cells, none were. For XP cells treated either in S or G_1 25% of the premutagenic lesions were located in the transcribed strand. Using UvrABC excinuclease to incise DNA at the site of adducts, followed by Southern blotting, and probing with strand-specific *HPRT* cDNA probes, we showed strand-specific repair of BPDE adducts. Sequence analysis showed that if BPDE-treated cells were allowed time for repair before S, the relative mutant frequency at site 212 and 229 of the *HPRT* gene increased significantly. No such change was seen in XP cells, suggesting that the increase was the result of inefficient repair at sites 212 and 229. To test this, we adapted ligation mediated polymerase chain reaction amplification, coupled with UvrABC excinuclease, to measure the rate of removal of BPDE adducts from individual sites. The results showed that the rate of repair at individual sites in exon 3 varied significantly and was slowest at nt 212 and 229, strongly supporting the hypothesis that inefficient repair is involved in mutation hot spots. (DHHS Grants CA21253 and CA56796.)

5 OXIDATIVE STRESS AND GLUTATHIONE REDOX STATUS IN MECHANISMS OF DEVELOPMENTAL EMBRYOTOXICITY

C Harris. *Toxicology Program, Dept. of Environmental and Industrial Health, University of Michigan, Ann Arbor, MI*

Chemical agents known to cause persistent anatomical and/or functional birth defects, have also been known to generate free radicals and reactive oxygen species (ROS). The uncontrolled production of ROS or free radicals, coupled with inadequate antioxidant defenses, creates conditions of oxidative stress which have been proposed as the primary insult through which many teratogenic lesions may be produced. Reactive oxygen species are reported to regulate embryonic development by providing signals for cell prohforation, differentiation and the initiation of apoptosis. Glutathione (GSH), low molecular weight thiols and related enzymes comprise a major antioxidant and radical scavenging defense system in most cell types. Glutathione also serves an important role in maintaining the proper cellular redox environment for control of normal gene expression and regulation of biochemical functions. It is probable that the disturbances in redox and antioxidant status that occur in response to chemical insult and environmental extremes are sufficient to alter genetic and biochemical functions and disrupt development. This symposium will explore the role and function of GSH status in the preimplantation and postimplantation embryo with respect to chemically-induced oxidative stress and describe several biochemical and molecular consequences resulting from the altered redox state. Phenytoin embrytotoxicity will be reviewed with respect to the ability of the conceptus to metabolize the compound to free radicals, the role of GSH in mediating this response, and the cellular and molecular alterations elicited by exposure. The session will conclude by evaluating the connection between ROS, cellular antioxidant functions, and control of programmed cell death involving products of the bcl-2 gene family.

6 GLUTATHIONE STATUS AND CONSEQUENCES OF CHEMICALLY-INDUCED OXIDATIVE STRESS IN THE PREIMPLANTATION MOUSE EMBRYO

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To understand the regulation of glutathione redox status and the mechanisms functioning to protect the embryo from chemically induced oxidative stress, tertiary-butyl hydroperoxide (tBH) was used to induce the oxidation of embryonic glutathione and diethyl maleate (DEM) was used to deplete embryonic glutathione. The concentration of reduced glutathione (GSH) in the embryo decreased 90% during normal development of the oocyto to the blastocysl. After 15 min exposure to 13.2 μM tBH, GSH levels dropped and oxidized glutathione (GSSG) levels increased. By one hour in culture, GSH levels had returned to normal in both two-cell and blastocyst stage embryos and this recovery was blocked by inhibition of glutathione reductase with 1,3-bis(2-chicro-ethyl)-1-nitrosourea. Two-cell stage embryos were unable to recover their GSH levels after depletion by DEM but blastocyst stage embryos completely recovered their GSH by 5 hours. Addition of L-buthionine-(S,R)-sulfoximine to the medium, to inhibit de novo synthesis of GSH, or elimination of cystine from the medium prevented recovery of GSH levels in the blastocysts. Chemically induced oxidation or depletion of GSH decreased the percentage of embryos that exhibited further development in vitro. The mRNA for γ-glutamylcysteine synthesis was detected in blastocyst stage embryos using reverse transcriptase-polymerase chain reaction but it was not detectable in the moruia stage embryo. These results indicate that the preimplantation embryo has considerable capacity to reduce GSSG but the cleavage and moruia stage embryo are unable to synthesize GSH de novo, apparently due to lack of expression of γ-glutamylcysteine synthetase. Studies suggest that glutathione related protective mechanisms have a critical role in regulating programmed cell death in the preimplantation embryo. The expression of genes for glutathione synthesis are developmentally regulated and may play a crucial role in the regulation of differentiation and apoptosis in the preimplantation embryo. In addition, the lack of GSH synthesis in the early embryo may make it susceptible to adverse effects of toxicants that deplete glutathione in vivo.

7 CHARACTERISTICS OF GLUTATHIONE REDOX AND ANTIOXIDANT STATUS IN POSTIMPLANTATION RAT EMBRYOS: RESPONSE TO OXIDATIVE STRESS

C Harris, E Lee, R Hiranruengchok, T L McNutt, S J V Larson, S Akella and B A Thorsrud. *Toxicology Program, Dept. of Environmental and Industrial Health, University of Michigan, Ann Arbor, MI*

Chemical exposure can result in the generation of free radicals and reactive oxygen species (ROS) in tissues of the developing embryo and its associated extraembryonic membranes. The extent of oxidative damage elicited by test chemicals such as diamide, alloxan, lindane and t-butylhydroperoxide depends ultimately on the specific cells capacity to respond to the changes elicited and to rapidly reestablish homeostasis. Administration of these agents to organogenesis-stage rat conceptuses (GD 10-11) in whole embryo culture results in the oxidation of glutathione to its disulfide and results in significant (2-16 fold) increases in glutathione-protein mixed disulfide formation. The latter is highly correlated with changes in thiol-sensitive processes such as decreased glycolytic activity (lactate production), changes in pentose phosphate shunt activity, altered pyridine nucleotide redox status and inhibition of pinocytosis. Antioxidant enzymes (glutathione peroxidase and glutathione disulfide reductase) are found within the developing conceptus in patterns where activities are high in tissues resistant to teratogenic lesions (heart and VYS) and low in sensitive tissues (head and trunk). The capacity to restore GSH lost through oxidation or adduct formation, especially via de novo synthesis, follows similar patterns in terms of cell resistance and sensitivity. Other important developmental events such as gene expression, cell proliferation, differentiation and programmed cell death may be influenced by GSH redox status, and the capacity to respond to conditions that produce oxidative stress.

8 REACTIVE OXYGEN SPECIES, MOLECULAR TARGET OXIDATION AND THE p53 TUMOR SUPPRESSOR GENE IN CHEMICAL TERATOGENESIS

P G Wells. *Facility of Pharmacy & Dept. of Pharmacology, University of Toronto, Toronto, Ontario, Canada*

The teratogenicity of numerous xenobiotics, including benzo[a]pyrene (B[a]P), phenytoin and related compounds, may be due in part to embryonic bioactivation to reactive free radical intermediates, catalysed by prostaglandin H synthase (PHS) and/or lipoxygenase (LPOs). If not reductively detoxified by glutathione (GSH), the free radical intermediates either directly or indirectly, via the formation of reactive oxygen species (ROS), may oxidise embryonic molecular targets such as DNA, protein and lipid, thereby initiating teratogenesis. For several xenobiotics, a free radical intermediate has been demonstrated directly in vitro by electron spin resonance spectrometry, and in the case of phenytoin, in vivo by the 3-hydroxylation of salicylates. In rodent models in vivo and/or in embryo culture, most of the cytoprotective enzymes that inhibit such oxidative stress also appear to be embryo protective, including GSH reductase, GSH peroxidase, superoxide dismerse (SOD), catalse and glucose-6-phosphate dehydrogenase. Similarly, murine phenytoin teratogenicity is reduced by antioxidants (GSH, caffeic acid, vitamin E), free radical spin trapping agents (phenylbutylnitrons) and the iron chelator deserferoximine. B[a]P, phenytoin and thalidoxide, as well as related compounds, oxidise GSH to GSSG, and/or oxidise embryonic lipid, protein and DNA in vivo and/or in embryo culture, the latter of which are inhibited by SOD or catalse. DNA may be a particularly relevant target, since the terrologic potency of phenytoin and related compounds in rodents correlates with their ability to oxidise DNA in an in vitro peroxidase bioactivating system. More directly, transgenic mice deficient in the p53 tumor suppressor gene, which facilitates DNA repair, are more susceptible to the teratogenicity of both B[a]P and phenytoin. These results suggest that ROS-mediated molecular target oxidation and DNA repair are important in chemical teratogenesis. (Support: Medical Research Council of Canada; Hospital for Sick Children Foundation of Toronto).

9 PRODUCTS OF THE BCL-2 GENE FAMILY, ANTIOXIDANT FUNCTION AND THE CONTROL OF PROGRAMMED CELL DEATH

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Sponsor: C Harris

Programmed cell deaths occur at many stages of normal development and maintenance of homeostasis in renewing tissues. Many of these cell deaths have characteristic features of apoptosis. Apoptotic cell deaths utilize a death mechanism intrinsic to the cell and are under the control of conserved positive and negative regulators. These include bcl-2 and bcl-2-like proteins that are found at mitochondrial and other intracellular membrances and act as general inhibitors or promoters of apoptotic death. Although a diverse set of triggers can initiate apoptosis, there is evidence for a common pathway in apoptosis that is regulated by bcl-2 and related proteins. We have identified oxidative stress as a general feature of apoptosis, as evidenced by the ability of antioxidants to inhibit cell death and the detection of lipid peroxidation at early time points after apoptotic stimuli are delivered. Protection of cells by bcl-2 expression correlates with increased resistance to oxidative stress, suggesting that bcl-2 may suppress endogenously generated oxidative stress at sites of generation such as mitochondria. Models will be presented for endogenous generation of oxidative stress during apoptosis, involving mitochondrial dysfunction and aberrant control of normal oxidative signal transduction pathways.

10 SCIENTIFIC CHALLENGES IN PESTICIDE RISK ASSESSMENT

J M Bankowska and M J DiBartolomeis. *Office of Environmental Health Hazard Assessment, Cal/EPA, Berkeley, CA*

In this presentation, we discuss emerging scientific issues pertaining to pesticide risk assessment. Health risk assessment provides estimates of the actual health hazard resulting from occupational and consumer exposure to chemicals, and therefore plays a fundamental role in the decision-making process relating to the use of pesticides. Limitations in the current methodology, the availability of data, and the quality of available data add varying degrees of uncertainty to these evaluations. In 1993, a National Academy of Sciences (NAS) report discussed some improvements in the assessment of risks to

children and infants from dietary exposures to pesticides. However, implementing these recommendations has raised several difficult issues related to the state of scientific knowledge, the adequacy of risk assessment methodology, and the interpretation of risk assessment results. We describe several scientific challenges facing risk assessors, including: assessment of risks resulting from exposure to multiple pesticides with the same mechanism of action, assessment of potential for neurotoxic effects other than cholinesterase inhibition, assessment of potential for immunotoxic effects, and exposure assessment for more sensitive populations.

11 IMPROVING RISK ASSESSMENT AND RISK MANAGEMENT IN FEDERAL REGULATORY PROGRAMS

G Charnley and G S Omenn. *Commission on Risk Assessment and Risk Management, Washington, DC*

The Risk Commission was mandated by Congress to consider various issues related to how human health risk assessments are performed and, most importantly, how the results of human health risk assessments are used in regulatory decision-making at congressional, federal, state, and local agency levels. Our undertaking is focussed on the five aspects of our mandate: uses and limitations of risk assessment in decision-making; appropriate exposure scenarios; uncertainty and risk communication; risk management policy issues; and consistency across agencies. The Commission will issue a draft report in March 1996, for public comment, on such issues as the use of "bright lines" for environmental regulation; sources of inconsistencies in risk-assessment and risk-management practices among agencies; how the uncertainty associated with risk estimates can be communicated in a useful way to regulators and the public; how communities, industries, environmental organizations, and other stakeholders can play an effective role in environmental regulation; how comparative-risk assessment can be used to set regulatory priorities; and how peer review and environmental justice and equity considerations should influence decision-making. The Commission also will comment on the recommendations of the 1994 National Research Council report *Science and Judgment in Risk Assessment*. Preliminary conclusions: (1) good risk assessments can improve regulatory decisions, but do not guarantee good regulatory decisions; (2) risk assessment results must be integrated with political, social, legal, economic, engineering, and cultural considerations to make decisions about the need and methods for risk reduction; (3) no single "best estimate" of risk can be used in all situations; (4) while qualitative statements describing critical uncertainties in a risk assessment are essential, routine use of current formal mathematical approaches to characterizing uncertainties are unlikely to enhance risk communication. The Commission will recommend a five-step framework that can be used to guide decisions about managing and reducing risks. We welcome inputs from members of the SOT.

12 KEY CONCEPTS IN APPLYING TOXICITY DATA TO DETERMINE FOLLOW-UP PUBLIC HEALTH ACTIONS AT HAZARDOUS WASTE SITES

D R Brown, W Webb, H Hansen and M L Lichtveld. *Public Health Practice Coordination Group and the Division of Toxicology, Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta, GA 30333*

Chronic and sub-chronic toxicity in persons exposed to low levels of environmental contaminants at hazardous waste sites is a public health concern. Toxicity data are essential to determine appropriate public health actions. ATSDR's public health assessment process includes a comprehensive analysis of each site by a multidisciplinary panel of public health professionals. All information including chemical exposures levels and human health conditions are analyzed by the panel to determine specific follow-up. Questions concerning the link between human health concerns and plausible toxic actions are key to the analysis. Specific toxicity questions were identified at 5% of the over 800 sites evaluated. These were referred to ATSDR's Division of Toxicology for assistance in determination of follow-up. The referrals were analyzed to identify key concepts to public health application of toxicology. Each referral is based on a specific health concern in an exposed population. Five types of site specific contaminant related specific questions were identified for 47 different substances. These were 1) basic dose response information on chemical intermediates; 2) information to assess plausibility of links between a chemical exposure and reported health condition; 3) advice in the absence of minimal risk levels for chemicals present in air, soil, water or biota; 4) specific research requests to address exposures to mixtures, degradation pathways, biomarkers, or bioaccumulation; 5) toxicity information to support epidemiologic studies. The insight derived from these questions indicates that effective public health interventions require a detailed understanding of both the actions of chemicals at low doses and the demographics of at risk populations. The findings further suggest critical new research questions to be addressed to support health interventions at hazardous waste sites.

13 PRIORITIZATION SCHEME FOR EVALUATING RISK ASSESSMENT ISSUES

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The risk assessment (RA) methodology developed by EPA for Superfund is the most commonly used approach for chemical RA despite lack of major revision since 1989. While there are advocates of improvements in many aspects of RA, there is little consensus among the scientific or regulatory communities about effective and efficient means to modify the existing methods. A means to prioritize issues in risk methodology, based on the significance of realizing an improvement in the method and the potential to achieve an improvement, would be useful as limited resources are available for updating risk methodologies. A ranking scheme was developed scoring areas for improvement based on 11 factors — 1) state of development, 2) ease of standardization, 3) availability of current EPA guidance, 4) regional variability, 5) conflicts with state guidance, 6) availability of standard techniques for application, 7) adequacy and acceptance of scientific basis, 8) technological requirements, 9) impact on RA cost, 10) impact on RA conclusiveness, and 11) impact on choice of remedial actions. A sample analysis indicated that high priority topics, those that could be addressed relatively easily and yield significant improvement, include guidance on site-specific land uses, standard exposure pathways for land uses, and considering detection frequency for analytical samples. Topics with low priority include tissue residue analysis, statistical treatment of background samples, and development of cleanup goals for multiple-source contamination. This scheme allows RA issues to be ranked based on their potential to improve the process and provides a rational approach for prioritizing methodology revision efforts.

14 TOXICITY RANKING SCREENING METHODOLOGY

B S Mintz[1], J C Riddle[2]. [1]*US EPA, Office of Water, Washington DC;* [2]*Life Systems, Inc., Cleveland, OH.* Sponsor: Edward V. Ohanian

In order to help set national priorities for research, guidance and regulation development for drinking water chemical contaminants based on risk, a toxicity ranking screening method has been developed. This method was developed by reviewing, selecting and adapting components from over 50 existing ranking methods. The goals in developing this method were to use parts of previously developed and reviewed methods, to be able to apply the method to chemicals with limited toxicity data, to provide a measure of the quality and completeness of the toxicity data for each chemical, to permit the use of readily available secondary sources for toxicity data input, to promote false-positive over false-negative results, and to have flexible and adaptable decision criteria. In order to score and rank chemicals based on toxicity concerns, the method uses potency data, severity of effect information and weight-of-evidence classifications for different toxic effects. Although this method was developed specifically to assist in prioritizing activities for drinking water contaminants, the method can be widely applied to any situation that requires ranking chemicals based on toxicity or potency concerns.

15 CATEGORICAL *VERSUS* NARRATIVE CARCINOGEN IDENTIFICATION SYSTEMS

A. G. Salmon, M. Sandy, L. Zeise. *RCHAS, OEHHA, Cal/EPA, Berkeley, CA*

Hazard identification for carcinogens serves both as a summary of existing scientific information and to provide risk managers with the information necessary to make public health protective decisions. Agencies such as IARC, US Environmental Protection Agency, and California (Dept. Health Services or Cal/EPA) have historically utilized categorical hazard identification systems to classify carcinogens. These categorical systems have relied primarily upon epidemiological and rodent bioassay data, with only secondary consideration of supporting data (*e.g.* short-term tests, structure/ activity relationships, mechanistic data). US EPA recently proposed a narrative approach to carcinogen identification as an alternative to a categorical system. The proposed narrative system would depend explicitly upon a "weight of evidence" approach. Here we compare the two approaches to each other and to what

might happen in real cases, using as examples 2,6-dinitrotoluene, chloral hydrate, and fumonisin B_1. The classification of 2,6-dinitrotoluene by either approach is straightforward, in comparison with chloral hydrate and fumonisin B_1, which exemplify the situation where some evidence in animals is accompanied by supporting data (from genotoxicity and γGT + liver nodule induction experiments respectively). Neither of the latter two agents met the criteria for identification as carcinogens when evaluated recently by the Proposition 65 Carcinogen Identification Committee, but both agents nonetheless raised both scientific and public health issues of sufficient importance for that Committee to direct specific comments to the National Toxicology Program and US Food and Drug Administration.

16 PROPOSAL FOR A COLLABORATIVE PROGRAM OF TECHNICAL COMMUNICATIONS; HEALTH SCIENCES RESEARCH AND TRAINING; AND HEALTH EFFECTS PREVENTION FOR AGRICULTURAL CHEMICALS IN THE MIDDLE EAST

D F Pena and K M Abdo. *NIEHS, RTP, NC.* Sponsor: H B Matthews

As part of the Middle East Peace process, NIEHS is conducting a preliminary hazard assessment of agrochemical use in the Middle East. The project is to include participation from technical experts from Egypt, Jordan, Israel, West Bank (Palestine) Territory and NIEHS. The project has six main objectives: 1) Establish an infrastructure for communication between technical experts 2) To assess the analytical and clinical capacities in each locality 3) To identify existing agrochemical risk reduction programs 4) Determine the types and quantities of agrochemicals used in each locality 5) Identify target populations, their exposure opportunities and exposure routes and 6) Develop intervention programs. The project is to foster communication and trust between participants while addressing a shared environmental health issue.

17 IN VIVO AND IN VITRO REACTION OF 2,4- AND 2,6-TOLUENE DIISOCYANATE WITH GUINEA PIG HEMOGLOBIN

B W Day, R Jin, and M H Karol. *Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA*

Previously, we reported the detection of toluene diisocyanate (TDI)-hemoglobin (Hb) adducts in erythrocytes from guinea pigs exposed to TDI vapor using immunochemical methods. In this study we further investigate the reaction of TDI with hemoglobin and compare *in vivo* and *in vitro* products. Synthetic Hb adducts of 2,4- and 2,6-TDI were individually prepared, each at a Hb tetramer to diisocyanate ratio of 1:0.9. Hb samples were subjected to C_4 HPLC chain/heme separation with UV detection. Collected LC fractions were analyzed for immunoreactivity using an antiserum prepared to a TDI-protein conjugate, and by pneumatically-assisted electrospray mass spectrometry (ionspray MS). Immunochemical analyses indicated a difference in chain specificity by the two isomers. Ionspray MS revealed the presence of adducts not recognized by the antiserum, but was unable to detect adducts in some of the fractions that were immunoreactive. Carbamoylation adducts, consisting of 1 TDI molecule with 1 isocyanate group hydrolyzed to an amino group were seen by MS with each isomer and with each globin chain. Ionspray MS analysis of the Hb isolated following *in vivo* exposure of animals to 2,4-TDI indicated carbamoylation products with both the α and β chains in which one of the two original isocyanato groups had been hydrolyzed to the amine. We also detected an amine-nitroso adduct on the α chain in the *in vivo* sample. These results indicate that at least one of the isocyanato moieties (or its masked equivalent) of 2,4-TDI survives passage through the lung, into the serum, and through the erythrocyte membrane to form an adduct with Hb. Supported by NIEHS ES05651.

18 IN VIVO ROLE OF INTERLEUKIN-1α IN SKIN IRRITATION INDUCED BY TRIBUTYLTIN

E Corsini, A. Bruccoleri, M Marinovich, and C L Galli. *Laboratory of Toxicology, Institute of Pharmacological Sciences, University of Milan, Milan, Italy*

The biocidal agent tributyltin (TBT) is used mainly in wood preservation and marine antifouling paints. TBT salts are well known skin irritants both in human and rodents. Skin irritation involves resident epidermal cells, fibroblasts of dermis, and endothelial cells as well as invading leukocytes interacting with each other under the control of a network of cytokines and lipid mediators. Because of their anatomical location, a role for cytokines, especially IL-1α, produced by keratinocytes has been proposed. The purpose of this study was to investigate the role of interleukin-1α (IL-1α) in TBT-induced irritation in mice. The ears of Balb/c mice were painted with different amounts of TBT chloride (67–536 nmol in acetone) or with acetone alone. Two hours later there was a dose-related production of IL-1α along with ear swelling and accumulation of skin water, all of which were partially prevented by intraperitoneal injection of antibody against murine IL-1α. By RT-PCR we showed that the neutralizing antibody also partially prevented TBT-induced *in vivo* IL-6 expression but no TBT-induced TNF-α expression, suggesting a paracrine effect of IL-1α on IL-6 production but not on TNF-α expression and indicating that other inflammatory mediators are involved. Regarding the mechanism of action, it has been shown that triorganotins bind to a component of the ATP synthase complex, disturb the proton gradient and inhibit mitochondrial ATP synthesis. Therefore, we hypothesize that TBT diverts electrons from the respiratory chain into the formation of reactive oxygen intermediates, known to be involved in activation of transcription factors and production of cytokines.

19 ARSENIC MODULATION OF CYTOKINE CROSS-TALK IN NORMAL HUMAN KERATINOCYTES

D R Germolec and M I Luster. *Environmental Immunology and Neurobiology Section, NIEHS, RTP, NC*

Previous studies in our laboratory have demonstrated alterations in gene expression and secretion of growth factors, including GM-CSF and TGF-α in primary human epidermal keratinocytes cultured in the presence of sodium arsenite. To explain how arsenic affects cytokine and paracrine responses in keratinocytes using GM-CSF, TGF-α and IL-1α. Treatment with TGFα resulted in a significant increase in GM-CSF mRNA expression, with little or no effect on its own, IL-1α, or TGFβ1 gene expression. In an inverse dose-response, law doses only of TGFα caused a significant increase in TGFβ2 mRNA levels. Keratinocytes treated with IL-1α or GM-CSF demonstrated increased gene expression for GM-CSF, TGFα, TGFβ1 and TGFβ2. With the knowledge that keratinocytes exposed to sodiun arsenite demonstrate elevated levels of both GM-CSF and TGFα, we examined the effects of sodium arsenite on these regulatory pathways. Antibodies to GM-CSF and TGFα had little effect on the arsenic-induced increases in mRNA expression for these cytokines. However, GM-CSF secretion was significantly reduced after pretreatment with antibodies to TGFα. Sodium arsenite caused a significant decrease in TGFβ2 mRNA levels. Pretreatment with anti-GM-CSF completely protected against this effect, while pretreatement with anti-TGFα was only partially protective. These studies suggest that arsenic acts on keratinocytes by several mechanisms including direct effects on gene expression as well as through the modulation of cytokine regulatory networks.

20 ROLE OF IRON IN HYDROQUINONE- AND CATECHOL-INDUCED INHIBITION OF IL-2 DEPENDENT LYMPHOBLAST PROLIFERATION

Q Li and B M Freed. *Albany Medical College, Albany, NY.* Sponsor: D A Lawrence

Benzene metabolites hydroquinone (HQ), catechol and phenol exist in large quantities in cigarette tar, produced via pyrolysis of tobacco flavinoids. Both HQ and catechol inhibited human IL-2 dependent lymphoblast proliferation by >90% at 50μM without affecting viability. Phenol had no inhibitory effect up to 1mM. HQ decreased transferrin receptor (TfR) expression from 116.5 ± 25 (mean fluorescence intensity) to 37.5 ± 1.3, whereas catechol did not affect TfR expression. The inhibitory effect of 50μM catechol (but not HQ) was reversed by adding 100μM $FeCl_3$, suggesting that catechol acts as an iron chelator and inhibits cell proliferation through iron deprivation. The combination of 20μM HQ and catechol slightly enhanced their inhibitory effects, whereas addition of phenol did not potentiate the effect of either HQ or catechol. In summary, HQ and catechol inhibit IL-2 dependent lymphoblast proliferation by interfering with iron metabolism.

21 ASSESSING THE MODULATORY EFFECTS OF LEAD (Pb) ON GENE EXPRESSION IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) BY mRNA DIFFERENTIAL DISPLAY

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There is evidence to show that Pb has the ability to enhance and suppress gene expression in vivo. In order to shed some light on the molecular mechanisms

of Pb modulating human immune function, the mRNA differential display technique was employed to identify the genes that can be induced by Pb in PBMC. Total cellular RNA was isolated from medium- or 50 μM Pb-treated PBMC (3 x 10^6 cells/mL) from four individuals after 24 h incubation, and the RNA samples were reverse transcribed with oligo-dT primers ($T_{12}MA$, $T_{12}MC$, $T_{12}MG$, or $T_{12}MT$). Polymerase chain reaction (PCR) was done in the presence of the same oligo-dT primer and a second 5'-10mer. The PCR products from meidum- or Pb-treated samples of each individual were loaded on the sequencing gel side by side for comparison. Five bands which are consistently present in Pb-treated samples but not in controls in these individuals were cut out, reamplified, cloned and sequenced. One band was identified as human ribosomal protein L8; another band has the same sequence as an unpublished sequence from GenBank. The others are essentially novel. Supported by NIH grant ES03179.

22 CANNABINOID INHIBITION OF IL-2 EXPRESSION IN THE MURINE T-CELL LINE, EL4.IL-2

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Evidence for cannabinoid-related immune inhibition through a receptor-mediated mechanism has been widely established. The CB2 receptor subtype is the predominantly expressed form of the cannabinoid receptor in lymphoid tissues and negatively regulates adenylate cyclase. In order to investigate the role of CB2 in T-cells, the effects of cannabinoids on the cAMP signalling pathway in the murine T-cell lymphoma, EL4.IL-2 were characterized. Northern analysis of poly(A)-purified RNA demonstrated that both EL4.IL-2 cells and B6C3F1 mouse spleen express CB2 as a 4.0 kb fragment, but that CB 1 (6.0 kb) was detectable only in spleen. Southern analysis of mouse and EL4.IL-2 genomic DNA, when probed with CB2, showed the same banding patterns, indicating that the CB2 gene is similar in B6C3F1 mice and EL4.IL-2 cells. Treatment of EL4.IL-2 cells with either cannabinol, a CB2 selective ligand, or Δ^9-THC disrupted the adenylate cyclase signalling cascade by markedly inhibiting forskolin-stimulated cAMP accumulation which consequently led to a decrease in PKA activity and a decrease in the binding of transcription factors to CRE and AP-1 DNA motifs. Likewise, a marked inhibition of PMA plus ionomycin-induced IL-2 protein secretion which correlated to decreased IL-2 gene transcription was induced by both cannabinol and Δ^9-THC. These findings suggest that cannabinoid inhibition, through CB2, of signal transduction via the cAMP pathway induces T-cell dysfunction which leads to a diminution of IL-2 gene transcription. (These studies were supported by NIH grants DA07908 and DA09171).

23 CANNABINOID-MEDIATED DISRUPTION OF THE CAMP SIGNALING CASCADE: A MECHANISM OF IMMUNE SUPPRESSION

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Immune suppression, a well known effect of cannabinoid compounds, is mediated by ligand binding to cannabinoid receptors. CB1 and CB2 are members of the G-protein superfamily and negatively regulate adenylate cyclase. Based on this, the objective of the present studies was to investigate the effects of cannabinoid compounds on downstream components of the cAMP signaling pathway in mouse splenocytes. Treatment of splenocytes with either Δ^9-THC (3.2, 12, 22 μM) or CBN (1,5,10,15,20 μM) resulted in a dose-dependent inhibition of PKA activity at all concentrations. Electromobility shift assay demonstrated a time-dependent increase in protein binding to a CRE oligomer in nuclear extracts from forskolin-stimulated (50 μM) splenocytes was maximum at 60 min and rapidly declines by 90–120 min. Splenocytes treated with either Δ^9-THC or CBN (22 μM) demonstrated a marked inhibition of DNA binding at all time points suggesting a decrease in the activation of CREB/ATF transcription factors. Furthermore, protein binding to the CRE motif was inhibited in a dose-dependent manner by Δ^9-THC. Forskolin stimulation was also found to increase binding to a kB consensus sequence in a time-dependent manner with peak activity at 30 min. Additionally, binding activity to this motif was significantly inhibited by treatment with CBN (20 μM). These results suggest the mechanism of cannabinoid-induced immune suppression lies at the transcriptional level by inhibiting the activation of several PKA-regulated transcription factors. (Supported by NIH R01DA07908 and RO1DA09171).

24 POTENTIAL ROLE OF TYROSINE KINASES IN POLYCYCLIC AROMATIC HYDROCARBON-INDUCED CALCIUM ELEVATION IN HUMAN T CELLS

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Certain polycyclic aromatic hydrocarbons (PAHs) which suppress T cell function may do so by interfering with intracellular signaling mechanisms. Studies with a human T cell line (HPB-ALL) showed that immunotoxic PAHs, such as 7, 12-dimethylbenz(a)anthracene (DMBA) and benzo(a)pyrene (BaP) cause both immediate (3-min exposure) and sustained (4-hr) increases in intracellular calcium (Ca^{2+}), while PAHs with lower immunotoxicity, such as benz(a)anthracene (BA) and anthracene (ANT) produce immediate rises in intracellular Ca^{2+} which diminish by 4 hr. Early, but not late, PAH-induced rises in intracellular Ca^{2+} are largely blocked by inhibition of tyrosine kinase activity (Krieger *et al, Toxicol. Appl. Pharmacol.* 127, 268, 1994). In these studies, we used an ELISA-based colorimetric method to measure tyrosine kinase activity of HPB-ALL cell lysates incubated with PAHs for 10 min or 4 hr. We found that cell lysates had a dose-dependent increase in total tyrosine kinase activity after 10 min incubation with DMBA. In addition, 10 min incubations with DMBA, 3-methylcholanthrene (3-MC), benzo(e)pyrene, and ANT produced significant rises in total tyrosine kinase activity, and these rises diminished for both 3-MC and ANT after 4 hr. Western blotting techniques confirmed a drop in tyrosine kinase activity of DMBA and ANT-incubated human peripheral blood T cells after 9 hr incubation. These data suggest that PAH-induced early rises in intracellular Ca^{2+} may be tyrosine-kinase dependent for both immunotoxic and non-immunotoxic PAHs, whereas tyrosine kinases may not be involved in PAH-induced Ca^{2+} changes at later timepoints. Supported by NIH RO1-ES05495.

25 CADMIUM CHLORIDE AND VANADIUM PENTOXIDE INCREASE IL-8 GENE EXPRESSION AND PRODUCTION VIA PROTEIN KINASE C SIGNAL TRANSDUCTION PATHWAY IN HEP G2 CELLS

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Previous study has shown that Hep G2 cells can serve as a model to evaluate hepatotoxicity caused by agents which induce reactive oxygen species. Two such chemicals, cadmium chloride ($CdCl_2$) and vanadium pentoxide (V_2O_5), were examined. Oxidative stress was monitored by measuring interleukin (IL) -8 gene expression and production by ELISA and RT-PCR, respectively. IL-8 gene expression and production were increased in cells treated with 10 μM $CdCl_2$ or 0.5 μg/ml V_2O_5. The increase of IL-8 production was reduced or eliminated by antioxidants. Protein kinase C inhibitor, staurosporine, completely blocked $CdCl_2$- and V_2O_5-induced IL-8 elevation. These studies suggest that chemicals with oxidant activity may induce inflammatory cytokine via protein kinase C in the liver.

26 EFFECTS OF 2-ACETYLAMINOFLUORENE (AAF) ON PROTEIN KINASE C ACTIVITY (PKC) IN MURINE SPLENOCYTES CULTURE

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Treatment of 2-acetylaminofluorene (AAF) to the murine splenocytes culture produced a dose-related suppression of the lymphoproliferative response induced by lipopolysaccharide (LPS). Since protein kinase C (PKC) have been reported to be a key enzyme in the pathways leading to LPS-induced B-cell proliferation, effect of AAF on PKC activity was studied in BALB/c mouse splenocytes culture. Pretreatment of AAF to the splenocyte culture for 18 h caused a dose-related suppression of total PKC activity induced by LPS, while AAF had no effect on intracellular cAMP concentration induced by forskolin. Time course effect of AAF on PKC activity indicated that short-term treatment of AAF (10 min) to splenocytes culture significantly increased the total PKC activity. The increase of this PKC activity was due to the large enhancement of the membrane PKC level compared to control. Meanwhile, treatment of AAF for 60 min caused a decrease of total PKC activity. These results suggested that AAF might cause transfer of cytosolic PKC to the membrane at the initial stage, and then a progressive loss of PKC activity occured in the membrane. AAF also inhibited the binding activity of the transcription factor complex, NF-κB, whose LPS-mediated induction in splenocytes is known to be dependent on PKC. These results suggested that

immunotoxic mechanism of AAF might be linked to the depletion of PKC. (Supported by the grant from the Ministry of Science and Technology, ROK)

27 REACTION AND DIFFUSION CHARACTERISTICS OF CRYOIMMOBILIZED RECOMBINANT *E. COLI* BIOCATALYST IN REMEDIATION OF NEUROTOXIC ORGANOPHOSPHATES

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Organophosphate (OP) nerve agents and pesticides pose significant environmental and human health threats. The plasmid-borne gene that codes for organophosphorous hydrolase (OPH) in *Pseudomonas diminuta* has been cloned and genetically engineered to enhance OPH expression in *E. coli*. This strain has been immobilized in a polyvinyl alcohol cryogel. Diffusion and reaction analysis of the biocatalyst in the degradation of model compound pesticides paraoxon and parathion was performed to enhance mathematical modeling of a bioreactor system. The natures of the diffusional resistances and deactivation characteristics of the biocatalyst were investigated by comparing pure OPH enzyme, freely suspended cells and immobilized cells. The activation energy of pure OPH enzyme was determined to be 32.7 kJ/mol while the activation energy of freely suspended cells was determined to be 62.7 kJ/mol. Activation energies were determined for varying biocatalyst cell loadings and diameters to establish optimal biocatalyst characteristics for the system. The apparent binding affinity of the biocatalyst for paraoxon is an order of magnitude greater than that of pure OPH and only 20% higher for parathion. This research establishes a functional biphasic system for degradation of hydrophobic OP neurotoxins. This study demonstrates that the remediation technology will allow for efficient and cost-effective degradation of OP compounds.

28 RISK ASSESSMENT OF CHEMICALS IN FISH WITH INTEGRATION OF RISK REDUCTION AND RISK COMMUNICATION

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This work integrates the principles and methodologies in risk assessment, risk reduction and risk communication in dealing with an environmental contamination situation. Risk assessment of chemicals in fish (methylmercury, PCBs, pesticides) resulted in the issuance of a health advisory recommending consumption limits for certain fish species in San Francisco Bay. General guidelines on ways to reduce chemical exposures, including fish preparation and cooking methods, were also issued. A special task force including representatives from government agencies, environmental groups and the public was formed to address risk communication issues. The advisories were translated into Cambodian, Chinese, Korean, Spanish, and Vietnamese, and transmitted to interested parties. A survey was performed to examine the effectiveness of the signs posted at two fishing locations for communicating the health concerns on an earlier related advisory. The issues and results relating to risk assessment, advisories, warning sign posting, multi-lingual educational materials, and survey will be discussed.

29 FIELD VERSUS LABORATORY QUANTIFICATION OF ECOLOGICAL IMPACTS FOR USE IN ECOLOGICAL RISK ASSESSMENT: HEAVY METALS IN AN AUSTRALIAN SEAGRASS COMMUNITY

K Trowbridge and M Katona. *ChemRisk, a Division of McLaren/Hart Environmental Engineering, Cleveland, OH.* Sponsor: M Gargas

By providing the environmental context in which community change occurs, this approach supports a more comprehensive examination of ecological impacts in addition to conclusions regarding the ecological significance of impacts associated with individual activities at the ecosystem level. The ecosystem evaluated consisted of an Australian near-shore seagrass community (*Heterozostera tasmanica*) that is influenced by both regional (shipping and land-use) and localized (industrial) impacts. A Geographic Information System (GIS) combined with multi-variate statistical approaches to data evaluation supported the ranking of ecological impacts/effects and their likely causes. Local land-use, shipping, dredging, industrial and meteorological information was combined with biological, chemical and physical characterization of the seagrass community to address cumulative impacts (i.e., the overall health of the ecosystem) as well as the cause and effect relationships of impacts associated with individual activities. Traditional LC_{50} comparisons from laboratory studies indicated the potential for effects from heavy metals at levels below those measured in reference bay sediment. Field quantification of actual community conditions revealed that conclusion regarding ecological risk based upon single organism laboratory LC_{50} comparisons was overly conservative and often invalid.

30 ROLE OF THE TOXICOLOGIST IN PROJECT MANAGEMENT OF A REMEDIAL INVESTIGATION AND FEASABILITY STUDY

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Risk assessment (RA) is a critical component of a Remedial Investigation and Feasability Study (RI/FS) in order to establish remediation clean-up goals for the site. Up-front involvement of the toxicologists who will execute the risk assessment tasks of the RI/FS study is crucial; however, other RI/FS project management concerns (geology, engineering, sampling and analysis, data management) can overshadow or even be at odds with the toxicologists' professional input. It is often overlooked that the primary function of an RI report is to support a comprehensive risk assessment and feasability study. A case study demonstrates how toxicologists' input in ensuring data and information key to the RA (off-site and background sampling, exposure assessment, toxicity assessment) resolved a potential dilemma: the RI focused primarily on nitroaromatic explosive constituents; however, the RA indicated that PCBs (e.g., fish consumption) contributed >95% to on-site/off-site risks.

31 INDIRECT RISK ASSESSMENT: CASE STUDIES OF HAZARDOUS WASTE COMBUSTORS

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In November of 1994, the Environmental Protection Agency (EPA) issued its final Strategy for Hazardous Waste Minimization and Combustion. In the Strategy, EPA outlined the role of risk assessment in assuring the safe operation of hazardous waste combustion facilities. In accordance with the goals of the Strategy, the TNRCC performed indirect screening analyses for several facilities. The methodology employed was that outlined in the *Guidance for Performing Screening Level Risk Analyses at Combustion Facilities Burning Hazardous Wastes* (EPA, 1994). A tiered screening approach, allowing progression from a generic worst-case risk assessment to an increasingly more detailed site-specific risk assessment, was developed for applying EPA's methodology. Interactive spreadsheets, consisting of approximately 50 fate and transport equations and an additional 25 algorithms for estimating human health risks by indirect pathways, was developed for performing the screens. Exposure scenarios included adult and child residents, subsistence farmers (dairy and beef), and a subsistence fisher. Ingestion of soil and vegetables were evaluated for each scenario. In addition to soil and vegetables, the subsistence farmer was also assumed to consume beef and/or milk (as appropriate) while the subsistence fisher was assumed to eat fish. Preliminary results indicate that ingestion of mercury-contaminated fish by the subsistence fisher dominated the non-cancer hazard indices for all of the facilities evaluated. For two facilities, ingestion of benzo(a)pyrene-contaminated fish by the subsistence fisher accounted for the majority of the cancer risk. For the third facility, ingestion of bis(2-ethylhexyl)phthalate-contaminated milk by the subsistence farmer provided the largest contribution to the overall cancer risk. In conclusion, the results indicate that for each facility evaluated, the indirect cancer risks and the non-cancer hazard indices were below target levels of 1E-05 and 0.5, respectively.

32 THE PURIFICATION OF A TNT-REDUCING ENZYME

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TNT (trinitrotoluene) is a nitroaromatic explosive that has been in widespread use for many years in road construction, mining, and demolition. However, the largest user of TNT, as well as other explosives, has been the military. Surface water, ground water and land sites have been contaminated during the manufacture, storage, and use of TNT. TNT has been shown to cause liver and kidney damage, leading to the need for remediation of these contaminated

areas. In spite of the high cost associated with transportation of the contaminated material and the building and maintenance of incinerators, the most common method of remediation is incineration. Bioremediation has been shown to be a promising, less costly method of treatment. Aerobic and non-aerobic bacteria, as well as fungi, are being studied to find strains suitable for bioremediation. We are currently working with three different aerobic bacteria that have varying abilities to degrade TNT. The work reported here is the purification of a TNT-reducing enzyme isolated from a *Bacillus sp.* This enzyme shows a TNT-dependent NADPH oxidation. Kinetic studies have shown this to be a first order reaction for both TNT and NADPH. The enzyme has a relatively small K_m, and has much higher activity with NADPH than NADH. Based on enzymatic activities using TNT metabolites as substrates, the enzyme appears to preferentially reduce the NO_2 group at the 4 position on the ring.

33 RATIONAL ENZYME DESIGN: COMPUTER MODELING AND SITE-DIRECTED MUTAGENESIS AS TOOLS TO IMPROVE CATALYTIC ACTIVITY AND SPECIFICITY OF ORGANOPHOSPHORUS HYDROLASE

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Organophosphorus hydrolase (OPH) is a bacterial metalloenzyme which performs the first cleavage step in the bioremediation of neurotoxic organophosphate compounds. Significant improvement of OPH catalytic activity (kcat) and substrate specificity ($kcat/K_M$) for some chemical warfare agents (CWAs) and their analogues has been achieved through site-directed mutagenesis of histidinyl residues 254 and 257. Individual mutants show up to 18-fold improvement in activity against demeton-S, 4-fold improvement in activity against VX, and 33-fold improvement in activity against soman. Many mutants retain excellent catalytic activity and specificity for the wild-type enzyme's preferred substrate, paraoxon, despite the loss of one of the two moles of metal present in each mole of native enzyme. E-ray crystallographic coordinates of the native wild-type apoenzyme and modeling of important features at the active site suggest critical attributes that can be further modified to continue the rational design of this enzyme for bioremediation of pesticide contaminants and CWA stockpiles. (We acknowledge H Holden, The Enzyme Center, University of Wisconsin for providing x-ray coordinates to enhance this study.)

34 ENZYME BASED BIOSENSOR FOR DIRECT DETECTION OF ORGANOPHOSPHOROUS NEUROTOXINS

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The widespread use of organophosphorous (OP) compounds in agricultural and industrial applications and the demand for chemical nerve agent destruction pose an environmental challenge for detection of these OP neurotoxins. In order to ensure that these substances are not present at hazardous levels in food, ground water, and soil, analytical methods have been developed and modified for many years. A new biosensor for the direct detection of OP neurotoxins has been developed. Immobilized organophosphate hydrolase (OPH) or cryoimmobilized recombinant *Escherichia coli,* capable of hydrolyzing a wide spectrum of OP neurotoxins were used as biological transducers. The pH-based assay was evaluated as a direct function of the concentration of organophosphates. A dynamic range of paraoxon determination between 0.25 ppm to 250 ppm (10^{-6} – 10^{-3} M) was observed. The response time for the flow-through system was 2 minutes for enzyme based biosensor and 20 minutes for the cell sensor. Immobilized OPH retained activity for over two month at room temperature. Cryoimmobilized *E.coli* exhibited a stable hydrolytic activity for over four months under storage in K-phosphate buffer +4°C.

35 COMPARISON OF HUMAN HEALTH AND ECOLOGICALLY-BASED REMEDIATION GOALS FOR NITROAROMATIC EXPLOSIVES IN SOIL

R S Prann, B L Roberts, D Duh, D Boyadjian and N Luke. *IT Corporation, Somerset, NJ*

A remedial investigation conducted at a former U.S. Army explosives manufacturing site delineated the nature and extent of contamination, and established clean-up remediation goals that would be protective of both human health and the environment. The contaminants of primary concern in surface soil were the nitroaromatic compounds 2,4-dinitrotoluene, 2,6-dinitrotoluene, and 2,4,6-trinitrotoluene. Dermal contact and incidental ingestion exposure scenarios were used to establish human health risk-based clean-up criteria for these nitroaromatic compounds in soil. Earthworm 14-day acute toxicity test results were used in combination with food web exposure models to establish ecologically-based clean-up criteria for soils.

Soil clean-up remediation goals were comparable (less than an order of magnitude difference) for both human health-based and ecologically-based criteria at this site.

36 ADVANTAGES OF DERMAL EXPOSURE ASSESSMENT FOR CHARACTERIZING OCCUPATIONAL RISKS TO SOIL AND WATER CONTAMINANTS

B L Roberts, R S Prann, and N Luke. *IT Corporation, Somerset, NJ*

A risk screening protocol was required to evaluate potential occupational exposure to environmental media (soil, surface water) using a single exposure route/pathway to characterize and compare health risks/hazards at each of more than 60 sites. Hypothetical scenarios for occupational exposure to soil or water by dermal contact or incidental ingestion were evaluated to aid in selection of a realistic and conservative risk screening protocol. Dermal contact was selected as a better overall measure of occupational exposure to environmental media. The dermal exposure algorithms provided comparable or more conservative estimates of exposure (i.e., larger daily dose rates) than incidental ingestion algorithms for these media. The use of a fixed body surface area (25%) allowed comparison of Chronic Daily Intake (CDI) and calculated risks/hazards of constituents for dermal exposure to soil or water using uniform and defensible default values.

37 THE Cr(VI) REDUCTIVE CAPACITY OF HOUSEHOLD BEVERAGES: IMPLICATIONS FOR RISK ASSESSMENT

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Laboratory studies were conducted to determine how rapidly and completely hexavalent chromium [Cr(VI)] is reduced to non-toxic trivalent chromium [Cr(III)] upon contact with common beverages mixed with tapwater. Studies were performed with five common beverages (coffee, tea, orange juice, Kool-Aid, and powdered lemonade) spiked with either 10 or 50 mg Cr(VI)/L. The concentrations of Cr(VI) were measured at several time intervals for up to 4 hours. It was demonstrated that each of these beverages had the capacity to reduce a concentration $\geq$ 8 mg Cr(VI)/L within 15 minutes, with orange juice and coffee having the capacity to instantly reduce > 50 and 25 mg Cr(VI)/L, respectively. These findings indicate that many common household beverages are capable of reducing substantial quantities of Cr(VI) to Cr(III). When considered in conjunction with the fact that the reductive capacity of the gastric juice may exceed 50 mg Cr(VI) daily, it is likely that little or no Cr(VI) is systemically absorbed upon daily ingestion of household tapwater containing up to 5 – 10 mg Cr(VI)/L (a concentration at which the water is bright yellow). These findings have significant implications for risk assessments involving ingestion of household tapwater containing Cr(VI).

38 EFFECTS OF CHEMICAL INTERACTIONS ON BACTERIAL MUTAGENICITY

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Standard methodology for human health risk assessment assumes additive interactions among the contaminants of a complex mixture. However, chemical interactions may occur which may result in synergistic or antagonistic effects. The mutagenic response of three model compounds, benzo(a)pyrene (B(a)P), pentachlorophenol (PCP) and 2,4,6-trinitrotoluene (TNT), were tested individually and in binary and tertiary solutions, using the *Salmonella/* microsome assay with each of three bacterial tester strains (TA97a, TA98 and TA100). For all strains, B(a)P was mutagenic with metabolic activation. TNT was mutagenic without metabolic activation, and pentachlorophenol was inactive. In binary and tertiary solutions, pentachlorophenol had no effect on the mutagenicity of B(a)P or TNT, with or without metabolic activation. For strain TA97a, the mutagenicity of B(a)P with activation was slightly decreased in the presence of TNT; the mutagenicity of TNT without metabolic

activation was slightly decreased in the presence of B(a)P and PCP; and the mutagenicity of the tertiary solution (496 revertants/10 ug) with activation was lower than B(a)P alone (729 revertants/10 ug). For strain TA98, B(a)P mutagenicity with metabolic activation was inhibited by the addition of TNT; and the tertiary solution with metabolic activation was not mutagenic. Assays were also performed using titrations of B(a)P and TNT in varying concentrations, and using four concentrations of S9. (Supported by Grant No. GTD0293 from the USEPA Health Effects Research Laboratory, RTP, NC).

39 A RESEARCH PROGRAM TO ASSESS THE TOXICITY OF CHEMICAL MIXTURES OF PUBLIC HEALTH CONCERN

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A strategic plan for mixtures research consisting of trend analysis/ listing, assessment, and experimental research is being pursued at ATSDR. The thrust of the plan is to involve the laboratory investigator, the model developer, and the public health assessor in designing animal studies or test systems, and selecting doses and dosing regimens to limit experimental variables and obtain information critical for the site-specific assessment process. This approach is consistent with the parallelogram model linking in vitro, and in vivo laboratory studies with projected health outcomes (CIIT Activities 14:1–9). The HazDat database has been evaluated to identify the most commonly occurring combinations of the top 275 chemicals found at Superfund sites [The Toxicologist 15 (1):216]. The next step in the development of health effects assessment methods for combined exposures is the delineation of a representative subset of these mixtures [The Toxicologist 14 (1):545]. Preliminary assessment of certain binary pairs of this subset reveals that limited interaction data exist for several pairs: Pb and Cr, Pb and Cd, and Pb and benzene. For some pairs, such as the effect of PCBs on the toxicity of vinyl chloride (VC) or that of VC on PCBs, the impact of interactions is symmetrical (identical reciprocal effects). For others, the impact is asymmetrical (different), such as the effect of chloroform ($CHCl_3$) on the toxicity of benzo[a]pyrene (B[a]P) or that of B[a]P on the toxicity of $CHCl_3$. For yet other pairs, the data appear to be ambiguous, and at times even contradictory. It is therefore extremely important for such assessments to clearly describe limitations and uncertainties in a narrative, "weight-of-evidence" fashion.

40 MODEL-DEPENDENT CYTOTOXIC RESPONSES TO DEFINED CHEMICAL MIXTURES OF METALS

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Many descriptive terms and mathematical, graphical, and statistical models have been used to evaluate the toxicity of simple mixtures. It is not very easy to distinguish clearly the intrinsic differences, distinctions and limitations of these models when applied to characterizing interactive toxicity. A series of experiments, based on ray experimental design were performed to illustrate model-dependent consistencies and differences in assessment of interactive toxicity. Cultured murine renal cortical cells, target cells for Cd and Hg toxicity, were treated with 0 to 50 μM $CdCl_2$, $HgCl_2$, or defined binary mixtures of Cd and Hg as to bracket EC_{50}s estimated for each toxicant. The cytotoxicity was determined at 24 hr by Lactate dehydrogenase release. The data were analyzed graphically and mathematically by a (a) isobologam, (b) response surface, (c) linear, and (d) non-linear models to characterize the interaction. The isobolgraphic and response surface analysis suggested antagonism, while the linear and non-linear models concluded no interaction. This comparison illustrates the complexity of extrapolating conclusions between models, and difficulty of public health assessment from exposures to multiple chemicals in the environment. Supported by ATSDR U61/ATU 581481.

41 EVALUATION OF THE HISTOPATHOLOGICAL AND DEVELOPMENTAL EFFECTS OF HYDROCARBON MIXTURES IN THE JAPANESE MEDAKA FISH

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Spills, leaks, wastewater discharges, and other releases from industrial activities have resulted in contamination of groundwater, surface water, soil, and air with petroleum hydrocarbons. The potential impact of environmental exposures to mixtures of hydrocarbons represents a highly relevant human exposure and ecotoxicity problem. This research evaluates the use of the Japanese medaka (*Oryzias latipes*) as a risk assessment model for screening complex mixtures of hydrocarbons for toxicological effects, particularly cancer and developmental effects. Ambient exposure of medaka embryos to benzene and toluene separately resulted in lethality dose-response curves that fit a log-probit model. Medaka embryos that were exposed to ambient benzene concentrations greater than 120 mg/L developed at a slower rate and had reduced progression of heartbeat development. Ambient exposure of the medaka embryos to the carcinogen benzene (67.7 mg/L) resulted in development of clear-cell focus in the liver, a preneoplastic lesion, in some fish at six months post-hatch. Other histopathological lesions noted in fish exposed to benzene during embryological development were mesangial lysis in the kidney and fatty changes in the liver. Toluene was more toxic than benzene based on lethality, embryo development and heartbeat progression. Developmental effects occurred in medaka exposed to ambient levels of toluene greater than 11 mg/L. Initial mixture exposures with benzene and toluene indicated that the combined toxic effect (lethality) of these chemicals was additive.

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42 DEVELOPMENT OF A SITE-SPECIFIC HEALTH-BASED CLEANUP LEVEL FOR TOTAL PETROLEUM HYDROCARBON (TPH) CONTAMINATION IN SOIL

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The TPH parameter is a non-specific analytical indicator of the quantity of residual petroleum-related compounds which are present at a large number of contaminated sites. Historically, TPH has been largely ignored within the quantitative risk assessment process because of the lack of appropriate toxicity information. MADEP and ABB Environmental Services have jointly developed a health-based approach for evaluating residual TPH contamination, which has been endorsed by the USEPA National Center for Environmental Assessment and Region IV. The health-based approach requires fractionation of the petroleum hydrocarbons into chemical classes and carbon chain ranges, and identifies appropriate reference toxicity values for each of the identified fractions. Other compounds for which specific toxicity information are available are considered separately. This general approach has been applied to soil samples collected at a site where residual TPH contamination has resulted from the use of a wide variety of petroleum products. The analytical method employed for fractionation involved GC/MS fingerprinting of TPH in soil to generate compositional estimates of the chemical classes and carbon chain ranges present. Combination of the compositional estimates with the reference toxicity values results in the estimation of a health-based cleanup level for residual hydrocarbons in soil of 5000 mg/kg, based on an assumed future residential scenario and the use of standard risk assessment dose calculation equations. Other considerations, such as odor concerns and potential leaching to groundwater, are also addressed in the evaluation.

43 EVALUATION OF A COMPONENT-BASED RELATIVE POTENCY APPROACH TO CANCER RISK ASSESSMENT FOR EXPOSURE TO PAHS

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One approach for estimating cancer risk from exposure to mixtures of polycyclic aromatic hydrocarbons (PAHs) assumes that all carcinogenic PAHs detected in the mixture are equal in potency to benzo[a]pyrene (BaP), the only PAH for which there are quantitative oral or inhalation cancer risk estimates. In this approach, concentrations of carcinogenic PAHs in the mixture are measured and summed as BaP equivalents. Cancer risk is then calculated by multiplying exposure (in terms of BaP equivalents) by quantitative oral or inhalation unit risk estimates for BaP. However, the central assumption of potency equivalence is inaccurate because published dermal and parenteral studies in animals show that PAHs differ in cancer potency. From a review of published studies, "estimated orders of potency" (EOPs) were derived for 13 PAHs in comparison to BaP [EOP = 1] as follows: EOP = 1: dibenz[a,h]anthracene, dibenzo[a,e]fluoranthene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene; EOP = 0.1: benz[a]anthracene, benzo[b, j and k]fluoranthene, chrysene, cyclopenta[cd]pyrene, dibenzo[a,i]pyrene and indeno[1,2,3-cd]pyrene; EOP = 100: dibenzo[a,i]pyrene. These EOPs were used to predict tumor responses in several published studies in which animals were exposed to complex PAH mixtures (e.g., synthetic mixtures with 10–12 PAHs, coal-burning emission condensate, coal liquids). Comparison of predicted and observed tumor responses showed close agreement, underestima-

tion or overestimation depending on the assay data set. Discrepancies between predicted and observed responses are discussed.

44 THE EFFECTS OF JP-8 JET FUEL ON FEMALE SPRAGUE-DAWLEY RATS AFTER A 21-WEEK EXPOSURE BY ORAL GAVAGE

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The U.S. Air Force recently converted from JP-4 to a less volatile JP-8 jet fuel similar to commercial Jet A. A 90-day oral JP-8 study conducted by this laboratory using male Sprague-Dawley rats resulted in significant dose-dependent decrease in body weight, as well as gastritis, perianal dermatitis and the male rat-specific alpha-2-microglobin nephropathy. In the present study, female rats were dosed with neat JP-8 (0, 325, 750, 1500 mg/kg) daily by gavage for a total of 21 weeks (90 day oral study followed by gestation and lactation) in an effort to assess additional adverse effects which may be associated with prolonged exposure to this fuel. Results of this study revealed a significant dose-dependent decrease in body weights of the female rats. Significant organ weight ratio increases were also seen for the liver:body, liver:brain and kidney:brain weights. There were no changes in urological, hematological or clinical chemistry parameters. Corresponding histopathologic changes and increases in liver enzymes (ALT, AST) were not observed although there was an increase in liver weight. Significant pathological changes were limited to squamous hyperplasia of the stomach and perianal dermatitis.

45 EROD AND PROD ACTIVITY FOLLOWING EXPOSURE TO AROCLOR 1254 AND CADMIUM (CD) IN MALE AND FEMALE DEER MICE

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Sponsor: R. Dickerson

We previously reported on the effects of exposure to Aroclor 1254 (20 ppm) in feed and Cd (400 ppm) in water for days 1-20 and 15-20 respectively, both single and in combination to male and female deer mice (Taylor, et al 1995). Subsequently, the exposed animal liver samples, stored at -80 C were used to characterize EROD and PROD activity. Statistically significant ($p<0.05$) EROD activity was observed for both males and females following exposure to Aroclor 1254 and the combination of Aroclor 1254-CD. Furthermore, the EROD activity for the combined group seem to reflect the toxicity of Aroclor 1254 in both males and females. In addition, EROD activity of 2.8 and 2.7 times the control for the males and 4.48 and 4.18 times control for the females, were calculated for Aroclor 1254 and Aroclor 1254-CD combination, respectively. PROD activity was significantly elevated for the CD and the combination of Aroclor 1254-CD treated males only. PROD activity for the combined group seem to reflect that of CD. Further research is needed to fully characterize the toxicity of environmental contaminants both single and in combination in males and females. This work was supported by NIH, NIGMS grants GM08117, GM08387 and NIEHS ES04696.

46 EXAMINATION OF THE EFFECT OF METHANOL ON TRICHLOROETHYLENE HEPATOTOXICITY IN MALE F-344 RATS

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Acute exposure to inhaled methanol (MeOH) alone has no effect on body weight, relative liver weight or serum indicators of hepatotoxicity and produces no histopathological evidence of hepatocellular degeneration or necrosis. Prior exposure to inhaled MeOH potentiates the hepatotoxicity of carbon tetrachloride and chloroform. The mechanism underlying this interactive toxicity can be explained, at least in part, by increased hepatic CYP2E1 activity in rats exposed to MeOH. The objective of the present study was to examine the interaction of MeOH and trichloroethylene (TCE), a common environmental contaminant whose metabolism is mediated by CYP2E1. Adult male F-344 rats ($n=6$/group, ~70 days old) were exposed to either 0 or 5000 ppm MeOH by inhalation for 6 hr. On the day following MeOH exposure, the rats were exposed to 0, 500 or 1000 ppm TCE by inhalation for 6 hr. Hepatotoxicity was assessed the day following exposure to TCE. Exposure to either MeOH alone or TCE alone had no effect on body weight, relative liver weight, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and produced no histopathological evidence of hepatocellular degeneration or necrosis. Exposure to 5000 ppm MeOH + 500 ppm TCE or 5000 ppm MeOH + 1000 ppm TCE had no effect on body weight or relative liver weight. There was no evidence of hepatotoxicity in animals exposed to 5000 ppm MeOH + 500 ppm TCE, based on serum ALT, AST and hepatic histopathology. In rats receiving 5000 ppm MeOH + 1000 ppm TCE, serum AST and ALT were not increased, relative to MeOH alone or TCE alone. However, minimal centrilobular vacuolar degeneration was observed in the livers of all 5000 ppm MeOH + 1000 ppm TCE rats. The present data indicate that prior exposure to MeOH results in a small potentiation of TCE hepatotoxicity that is dependent on the concentration of TCE. (This abstract does not reflect EPA policy.)

47 USE OF THE DOSE ADDITION MODEL TO ASSESS THE INTERACTION BETWEEN THE WATER DISINFECTION BY-PRODUCTS CHLOROFORM ($CHCl_3$) AND BROMODICHLOROMETHANE (BDCM)

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$CHCl_3$ and BDCM are the two most prevalent chlorination disinfection by-products, occurring ubiquitously in treated drinking water. The potential human health risk posed by chemical mixtures is due to the possibility of synergistic (greater than additive) interactions. The additivity assumption may be tested by either response addition ($1+1=2$) or dose addition ($D_1/D_{x1} + D_2/D_{x2} = 1$ where D_{x1} and D_{x2} are isoeffective (equitoxic) doses and D_1 and D_2 are the concentrations in the mixture). Although response additivity has been used more frequently for noncancer health effects, the appropriateness of its use for two active chemicals has been questioned. The objective of this study was to use the dose addition model to assess interactive hepatotoxicity after concurrent exposure to $CHCl_3$ and BDCM given orally in an aqueous vehicle. Fischer-344 rats (~90 days old, $n=6$) were gavaged with various dosages of $CHCl_3$ or BDCM alone (based on prior dose-response curves) and mixtures of $CHCl_3$ + BDCM. Hepatotoxicity was assessed 24 hr later by serum alanine (ALT) and aspartate (AST) aminotransferase and sorbitol dehydrogenase (SDH). At 1.0 mM/kg, where $CHCl_3$ and BDCM were equitoxic, a 1:1 mixture (0.50 mM/kg $CHCl_3$:0.50 mM/kg BDCM) did not deviate significantly from additivity for ALT, AST and SDH. BDCM and $CHCl_3$ were also equitoxic at 1.5 mM/kg; a 1:1 mixture (0.75 mM/kg $CHCl_3$:0.75 mM/kg BDCM) did not deviate from additivity in one experiment although dosage ratios of 2:1 (1.0 mM/kg $CHCl_3$:0.5 mM/kg BDCM) and 1:2 (0.5 mM/kg $CHCl_3$:1.0 mM/kg BDCM) were significantly less than additive at $p=0.05$, but not at $p=0.01$, in another. At 3.0 mM/kg, where $CHCl_3$ and BDCM were isoeffective for AST and SDH, nonadditivity was not detected for dosage ratios of 2:1 and 1:2. In sum, within the dosage range examined, synergistic interactions between $CHCl_3$ and BDCM were not found with the dose additive model. (This abstract may not reflect EPA policy).

48 METABOLITES IN RAT AND MOUSE URINE FOLLOWING ADMINISTRATION OF A MIXTURE OF [1, 2, 3-^{13}C]ACRYLAMIDE AND [1, 2, 3-^{13}C]ACRYLONITRILE: ANALYZED BY NMR SPECTROSCOPY

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Acrylamide (AM) and acrylonitrile (AN) are commonly used in the same facility to manufacture plastics and polymers. AM is a neurotoxin, genotoxin, reproductive toxicant, and carcinogen in laboratory animals. AN is carcinogenic in rats. Both AM and AN are metabolized by glutathione (GSH) conjugation and oxidation to reactive epoxides. The objective of this study was to evaluate the metabolism of each compound following administration as a mixture. An equimolar mixture of AM and AN (21 mg/kg and 16 mg/kg, respectively) was administered po to male F344 rats and B6C3F1 mice for four days. On day five, the animals received a mixture of the ^{13}C-labeled compounds. Urine was collected for 24 hr, and metabolites were analyzed using ^{13}C-NMR spectroscopy. Thirteen metabolites were detected directly in the urine and assigned to products derived from GSH conjugation with the parent compounds (AN or AM) and derived from GSH conjugation with the epoxides glycidamide and cyanoethylene oxide. Competition between AM and AN for GSH conjugation, cytochrome P450 oxidation, and hydrolysis is being assessed through quantitation of metabolites. These data will be

compared with metabolic profiles evaluated for rats and mice coadministered AM and AN for 1 day, or administered AM or AN for 1 day or 5 days. This study demonstrates the utility of using ^{13}C-labels and NMR spectroscopy for the analysis of the metabolism of mixtures.

49 CHEMICAL MIXTURES ENHANCE THE DERMAL BIOAVAILABILITY OF CHLOROFORM IN PIG

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Humans are exposed more often to chemical mixtures than to single compounds. The purpose of this study was to determine the effect of a chemical mixture containing chloroform, arsenic, toluene and phenanthrene on the dermal bioavailability of chloroform. An in vitro approach consisting of flow-through diffusion cell methodology was utilized on dermatomed female pig skin treated either with ^{14}C-chloroform alone or in the mixture. The maximum penetration rate for the mixture occurred later than for chemical alone (22 vs 5 min, respectively). However, the mixture doubled the total amount of radioactivity which penetrated into receptor fluid in comparison to chloroform alone. Although there was no change in the quantity of radioactivity remaining covalently bound to skin after both treatments, the total penetration was significantly increased when chloroform was applied in combination relative to chloroform alone. Therefore, the dermal bioavailability of chloroform is significantly higher for the mixture than for chemical alone. (Supported in part through funding from the Hazardous Substance Management Research Center and the New Jersey Commission on Science and Technology).

50 RISKY BUSINESS: LIVING IN A CHEMICAL WORLD

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This is the first of four units in our computer-based environmental health sciences curriculum for high school. The curriculum teaches basic biology and chemistry while incorporating principles of environmental health and toxicology. We use examples from toxicology (Toxic Connections) to help make science relevant to the student. Graphics and animation allow students to observe complex biological processes. For example, students can unwind a piece of double-stranded DNA to remove and examine its nucleotide components. Our curriculum is designed to promote active learning. Students are asked to play the role of the scientist, making observations of various phenomena (e.g., cell division) on a notepad that appears on the computer screen at certain prompts. This encourages students to learn by observation. Unit one, Basic Cell Biology teaches the structure and functions of the cell and its components. Topics covered in this first unit are: Structure and Function of Organelles; DNA, RNA, Protein; The Cell Cycle; and Mitosis and Meiosis. The DNA, RNA, Protein chapter links to a computerized version of the Ames Test. Once students learn the structure of DNA, they can learn how natural and man-made chemicals may alter DNA, affecting RNA, protein, and possibly human health. The three other curriculum units explore general physiology of the brain, GI tract, liver, and cardiovascular system; basic principles of toxicology; and the social, political, and public health aspects of assessing risks and alternatives, showing how daily decisions influence our own personal risks. (Supported by NIH grant R25 ES06938).

51 GROUP LEARNING MODELS CONSULTATION AND BEGINNING EXPERTISE IN A PROFESSIONAL TOXICOLOGY COURSE

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We previously found that students scored best on small animal neurotoxicology evaluations when tested initially in groups, and later as individuals. We now used a balanced incomplete block design to designate 15 randomly assigned groups to take 2 of 6 unannounced exams as groups and the remaining 4 exams as individuals. Groups scored higher than individuals in 4 of 5 exams given. Students previously group-tested demonstrated better understanding (ability to explain underlying mechanisms) on a later exam taken as individuals. We speculate that group validation of improved understanding is an initial step in developing toxicology expertise/mastery. Group scores did not predict increased individual performances on the later exam, even on questions that were similar to those the same students took in groups. Students selectively answering 70% of all possible points found the difficulty of treatment < diagnostic reasoning < explaining mechanisms. Groups demonstrated improved diagnostic reasoning more rapidly than individuals, recording higher scores for treatment and explaining mechanisms. This modeled future parameters for group consultations. [Supported by the State of Kansas and the US Department of Education, Fund for Improvement of Post-Secondary Education.]

52 INCORPORATING INTERNET RESOURCES INTO A MULTIMEDIA APPROACH TO ENVIRONMENTAL HEALTH CURRICULUM

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Toxicology and other environmental health curriculum (e.g., air quality, water quality, hazardous substances, etc.) must remain current and innovative in order to prepare students for the rapidly changing work place. Today's curriculum must utilize multimedia resources to provide access to the latest non-text information. Where once libraries were the only way to augment classroom lectures, Internet and software can now be used to enhance information access and problem-solving skills. Internet is the most economical way to bring problem-solving strategies into the curriculum. All students in each course obtain a university Internet account. NETSCAPE, gopher, and other search tools are then used to access Internet to find and use agency documents and databases (e.g., USEPA, OSHA, CDC, ATSDR), pollution modeling software, the federal register, demographic information, morbidity and mortality information, on-line abstracting services like the National Library of Medicine's Medline, meteorological information, agency and programmatic information, medical and toxicological terminology and acronyms, sampling strategies, and much, much more. They can also sign on to appropriate listservers (e.g., EPA-TOX, EPA-AIR, EPAFR-CONTENTS, etc.). Homework assignments and projects are designed to facilitate Internet expertise and to develop problem-solving skills to enhance lecture materials. Student feedback has been positive with many students anxious to explore this resource beyond classroom requirements. The utility of Internet is limited only by the imagination and creativity of the instructor.

53 PEDIATRIC BLOOD LEAD IN SOUTH CENTRAL LOS ANGELES

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We retrospectively reviewed 3679 pediatric records from the King/Drew Medical Center in South Central Los Angeles between January 1, 1991 and December 31, 1994 to describe blood lead levels and trends in children up to 18 years. Children tested were not specifically referred for lead exposure. 73.3% of the children were Latino, 24.7% were African-American and the remaining 2.0% other or unknown. Geometric mean blood lead level peaked at 6.7 μg/dl (0.32 μmol/l) between 2 and 3 years of age. There was a highly significant downward trend in blood lead level over the study period, amounting to 1.0 μg/dl. There was a significant seasonal trend amounting to 1.3 μg/dl, with lowest values in late winter and early spring, highest values in mid-summer. The seasonal trend was not correlated with seasonal averaged air lead levels of monitoring stations within and around the catchment area. Males had higher blood lead than females and Latino children were proportionally over-represented in higher blood lead categories compared to African-American children. Median family income of zipcode areas was highly negatively correlated with zipcode-grouped blood lead levels. Among Latino children 1–5 years of age, 20.2% exceeded 10 μg/dl blood lead, greater than expected from recently published NHANES-III preliminary results of comparable urban Latino children. As Latinos continue to move into deteriorated inner city areas recently vacated by more upwardly-mobile African-Americans, Latino children may replace African-American children as the group at greatest risk for non-occupational exposure to lead.

54 ZINC AND CADMIUM IN SOILS OF METROPOLITAN NEW ORLEANS

H W Mielke, Chris Gonzales, and M K Smith. *Xavier University of LA, New Orleans, LA.* Sponsor: H L Komiskey

The pattern of zinc and cadmium accumulation in the soils of New Orleans is the topic of this study. This study is based on a sample collection of 3,704 soils from metropolitan New Orleans. This is the same sample collection

that was used to describe the pattern of lead in New Orleans. The stratification of the soil samples was by census tract. All samples were collected from the surface 2.5 cm of the soil. The samples were collected in order to represent three residential environments: soils within 1 m of the street, soils within 1 m of the foundation, and soils from an open plot of land (vacant lot, large yard or park). The soil samples were taken to the laboratory where they were air-dried and sieved (USGS #10 sieve). The sieved samples were prepared for analysis by shaking 5 g soil and 25 ml of 1 M HNO_3 extraction solution for 2 hours. The extract was diluted by 10 making a dilution factor of 50. The analysis of trace metals was conducted with a Spectro Inductively Coupled Plasma Atomic Emission Spectrometer. The hypothesis for the current study is that zinc and cadmium follow the same pattern as Pb, that is highest in the inner-city with a steep decline in zinc toward the exterior of the city. The highest zinc levels are above 2000 μg/g. This amount of zinc is in a range expected to cause phytotoxicity. If so, zinc may be inhibiting the growth of ground cover in areas of the city that have the highest quantities of lead. (This research is supported by ATSDR grant U50-ATU398948-03).

55 ICP-MS MEASUREMENTS OF < 0.1 TO 1 μg/dL BLOOD LEAD CONCENTRATIONS IN CONTEMPORARY MARINE MAMMALS, WHICH APPROACH NATURAL PbB LEVELS IN PREINDUSTRIAL HUMANS

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Ambient blood lead concentrations of neonatal and suckling elephant seal (*Mirounga angustirostris*) pups are in the low part-per-trillion (0.1 to 1 μg/dL). These concentrations are below the analytical detection limit of graphite furnace atomic absorption spectrometry (GFAAS) and electrochemical (ASV) methods. However, they may be directly determined without preconcentration by ICP-MS following an acid digestion. This is accomplished with a Finnegan ELEMENT magnetic sector mass spectrometer, utilizing an ultrasonic nebulizer. The instrumental blanks for these analyses are at the parts-per-quadrillion level, and the procedural blanks are at least one order of magnitude lower than the sample concentrations. Consequently, these relatively low PbB concentrations, which approach the natural PbB concentrations of preindustrial humans, may be readily quantified.

56 THE USE OF A STABLE LEAD ISOTOPE METHODOLOGY TO EVALUATE THE EFFICACY OF HOUSEHOLD LEAD ABATEMENT FOR REDUCING SOURCES OF CHILDHOOD LEAD EXPOSURE

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Identification of lead hazards in housing are based largely on the lead concentrations of environmental samples (e.g., paint, dust, soil, water), which are used to evaluate the predominant source(s) and magnitudes of lead exposures to children. However, recent studies have indicated that elevated lead concentrations in dust and soil, etc. may not predictably account for the elevated PbB levels of children within those households, suggesting that dust or soil lead abatement may not always prove efficacious in substantially reducing the elevated lead exposures of resident children. The objective of this study is to use a state-of-the-art stable lead isotope methodology to evaluate the relative benefits of household lead abatement in reducing childhood lead exposures from identifiable sources of lead. Household environmental samples (dust, paint, soil and tap water) and venous blood samples from moderately lead-exposed resident children (PbB > 15μg/dL) are being collected prior to and again 6 months following household lead abatement for lead concentration and stable lead isotopic compositions analyses by TIMS. This study is being conducted with cooperation from the Exposure Prevention and Source Reduction section of the California Department of Health Services (CDHS) Childhood Lead Poisoning Prevention Branch (CLPPB). (Supported by a grant from the University of California Toxic Substances Research & Teaching Program).

57 ENZYMATIC METHODS FOR THE DETECTION OF ZINC SULPHATE BY SIMPLE CHROMATOGRAPHIC METHOD

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The present investigation was undertaken to analyze the zinc sulphate by a simple enzymatic method using paper and thin layer chromatography. Nearly eleven solvent systems, both organic and inorganic are tested for selecting a suitable solvent for the separation of and identification of zinc sulphate. The zinc sulphate is detected, separated and identified by sheep liver succinate dehydrogenase inhibition using 2-(4-idophenyl-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride (INT)-sodium succinate-N-methyl phenazonium methosulphate mixture as chromatographic for microthin layer chromatography. The results indicate that 2 μg of zinc sulphate can be detected by this method from various environmental soil and water samples. In conclusion the method described in the present study is highly recommended to establish an evidence of the existence of heavy metal compound.

58 DETERMINING THE BIOAVAILABILITY OF LEAD IN CONTAMINATED SOIL

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Determining the bioavailability of Pb in a diverse range of soils, allows scientifically-derived data to dictate appropriate remedies at these sites to reduce the risk of childhood Pb exposure to acceptable levels. This experiment ascertained the relative bioavailability (RB) of Pb in contaminated soils, by comparison with an orally administered Pb salt (Lead acetate = PbAc). Fifty-three male pigs (7.5- to 8.5-kg) fed a low-lead (< 0.2 ppm Pb) diet were randomly assigned to 1 of 11 treatment groups and dosed with PbAc or lead-contaminated soil for 15 days. Biological responses (area under the blood Pb [BPb] concentration-time curve [AUC] versus dose, and terminal liver-, kidney-, and bone-lead concentrations versus dose) to several dose levels of Pb from PbAc and lead-contaminated soil were measured. Lead in the test soil that was as bioavailable as Pb from oral PbAc was calculated using the average ratio of doses of Pb from PbAc and test soil that produced equivalent biological responses. Lead from soil containing 1600 ppm Pb had a relative bioavailability (i.e. relative to Pb from PbAc) ranging from 38% based on the AUC versus dose, to 20% based on calculations from bone-Pb loading versus dose. Similarly, Pb from soil containing 6300 ppm Pb had a RB ranging from 34% based on the AUC versus dose, to 22% based on calculations from bone-Pb loading versus dose. Site-specific knowledge of soil Pb bioavailability can be considered by risk assessors for use *in lieu* of EPA's default assumptions of 60% RB.

59 THE SKELETON IS AN IMPORTANT SOURCE OF ENDOGENOUS LEAD EXPOSURE IN HUMANS

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A stable lead isotope methodology has been used to evaluate the role of the skeleton as an endogenous source of lead to the circulation in humans. Bone and blood samples from 20 subjects receiving hip or knee joint replacements were analyzed for their lead concentrations and stable lead isotopic compositions (PbIC) by TIMS. Composite (3-day) fecal samples from 4 of the subjects were also analyzed to derive a measure of the amount and PbIC of current lead exposures and to compare to environmental estimates. Multiple trabecular and cortical bone samples were obtained from an autopsied individual to evaluate the isotopic heterogeneity of lead within the skeleton. Blood (1 – 9 μg/dL) and bone (0.5 – 24 μg/g dw) lead concentrations were generally low. This, in conjunction with the lead content of the fecal samples (3 – 28 μg/3 days) reflects exposures to low levels of environmental lead (e.g., < 10 μg Pb/day). Based on the stable lead isotope data, 11 subjects fit a simple 2-source (skeletal *vs* exogenous) model of lead exposure. Four subjects did not fit the model (possibly due to other unmeasured exogenous exposures), while 5 subjects neither clearly fit or did not fit the model. For the group (n = 11) who fit the model, it was found that the skeleton contributed approximately 61% (mean; range 31 – 79%) of the lead contained in the circulation. These data demonstrate (i) the significance of the skeleton as a source of lead to the circulation, and (ii) the utility of the stable isotope methodology for evaluating bone lead release. (Supported by NIEHS Grant #ES04850).

60 MERCURY LEVELS IN TARGET ORGANS OF FEMALE RATS FOLLOWING MULTIPLE DOSES OF MERCURIC CHLORIDE

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Sponsor: R R Dalvi

The acute and chronic toxicity of inorganic mercury in human have been studied extensively. Most of the mercury found in the environment is in inorganic forms. This study was designed to determine the levels of mercury in various organs following multiple doses of inorganic mercuric chloride in female rats (Sprague Dawley). The rats were given 0.0, 0.25, 0.50, 1.0, 2.0 and 4.0 mg/kg mercuric chloride (oral gavage) daily for fourteen days after three days of acclimation. At the end of the experiment, surviving rats were euthanized and tissue samples from their brains, gonads, hearts, kidneys, livers, lungs, pancreases and spleens were collected and analyzed for mercury content by cold vapor atomic absorption. At doses of 0, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/kg, mercury levels in kidneys were 0, 6.29, 9.01, 13.72, 14.64, and 17.59 μg/g respectively; while in the livers mercury levels were 0, 0, 0, 0.02, 0.70, and 1.56 μg/g, respectively. At all doses, mercury levels in the kidneys were significantly higher than those of livers. Mercury levels in the lungs, pancreases and spleens were undetectable at 0.25, 0.50, 1.0 and 2.0 mg/kg dosages of mercuric chloride. However, at the 4.0 mg/kg dosage, mercury levels in the lungs, pancreases and spleens were 0.10, 0.17, and 0.04 μg/g, respectively. At all doses except 4.0 mg/kg, mercury levels were undetectable in the brains, gonads, and hearts. (Supported by ATSDR grant no 5R51/ATR398004-01)

61 BLOOD LEAD IN PREGNANCY: STABLE ISOTOPES REVEAL CHANGES IN BOTH BONE LEAD AND CURRENT ORAL LEAD CONTRIBUTION IN A NON-HUMAN PRIMATE (Macaca fascicularis)

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Measurements of lead in blood (total and isotope ratios) were carried out before, during and after pregnancy in cynomolgus monkeys continually dosed with lead (nominally 1500μg/kgbw/day) for 12+ years. The administration of isotopically altered lead doses (i.e. with unique isotopic 'fingerprints') allowed us to deduce that an increase in blood lead during late pregnancy in some animals was mainly due to increased absorption (or reduced elimination) of oral lead. An increased flux of bone lead to blood lead was seen in 5 out of 6 animals during the second half of pregnancy, and is assumed to be related to the considerable mineral (calcium) demands of the developing fetal skeleton. The data also revealed other extended periods of bone lead mobilization in some animals, notably post partum. A physiologically-based kinetic model developed for lead in non-human primate pregnancy could reproduce the measured blood lead 'profile' by incorporating (a) an increased bone turnover rate during late pregnancy and (b) a progressive increase in fractional absorption of lead for those animals where lead isotope data had demonstrated changes in oral lead kinetics. That kinetics of lead under a steady state exposure in these non-human primates can apparently differ from animal to animal, is supportive of recent observations from humans, where, according to the individual, blood lead may increase or remain stable in late pregnancy. Supported by NIEHS Contract No.: NO1-ES-05285

62 ASSESSMENT OF DNA-PROTEIN CROSSLINKS IN LYMPHOCYTES OF INDIVIDUALS EXPOSED TO FORMALDEHYDE OR CHROMIUM

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DNA-protein crosslinks (DPC) were measured in lymphocytes of chrome platers, furniture workers and controls from Bulgaria in order to further evaluate a utility of DPC as a biomarker of human exposure. The chrome platers had significantly elevated levels of Cr in urine, erythrocytes and lymphocytes as compared to the control subjects. The largest differences between the Cr-exposed and control subjects were found in erythrocyte Cr concentrations which are considered to be indicative of Cr (VI) exposure. DPC correlated quite well with Cr in erythrocytes at low and moderate doses, but at high exposures such as among the majority of chrome platers, these DNA adducts were saturated at a maximal level. Occupationally unexposed subjects exhibited a significant variability with respect to Cr in erythrocytes, however, erythrocyte Cr levels showed a significant correlation with DPC in lymphocytes. Occupational exposure to formaldehyde among furniture factory workers did not effect DPC levels in peripheral lymphocytes. DPC measurements showed a low intraindividual variability and among both controls and exposed individuals their levels were not effected by smoking, age or weight.

63 EXCRETION OF METHYLMERCURY FOLLOWING CHRONIC INGESTION OF CONTAMINATED SEABASS

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The Wisconsin Division of Health investigated mercury exposure in a 40-yr old man, his 42-yr old wife and their 2.5 yr old son. At the time of our investigation, these individuals had blood mercury levels ranging from 37 to 58 ug/L (normal < 5 ug/L) and hair samples from the adults contained 10–12 ug mercury/g dry weight. A personal interview and home inspection failed to identify any occupational or household sources of mercury exposure. The family's diet included two meals of imported seabass per week. Two samples of the seabass obtained from the vendor on different days contained mercury concentrations of 0.5 and 0.7 mg/kg. Based on consumption estimates, the average daily mercury intakes for these individuals ranged from 0.5 to 0.8 ug/kg body weight. Six months after the family stopped consuming the seabass, blood mercury levels in this man and woman were 5 and 3 ug/L, respectively. Analysis of sequential blood samples confirmed that mercury elimination followed first-order kinetics with a half-life of approximately 60 days.

64 L-LINE X-RAY FLUORESCENT (LXRF) MEASUREMENTS OF BONE LEAD (Pb) IN VIVO: METHODOLOGICAL CONSIDERATIONS AND INSTRUMENT IMPROVEMENTS

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1) In 144 lead poisoned children, with BPb values above the median ≥11 ppm) and a BPb concentration ≥35 μg/dl, the Pb mobilization test (Pb-MT) outcome was predicted correctly in 83 percent of children. When age was entered as a variable, LXRF and BPb predicted the Pb-MT result in 90 percent. Thus, the toxicokinetics of Pb in cortical bone sampled by LXRF differs according to age. 2) We evaluated the speculative impact of skin thickness on LXRF values and, with BPb levels, to predict Pb-MT outcomes. Age, BPb and LXRF values were entered into multivariable analyses to yield a 2regression model (coefficient 0.381, $p < .001$). Next, skin correction factors were entered into analysis and a second regression coefficient was determined (0.372, $p < .001$). Thus, skin correction factors neither significantly modified the coefficients nor the statistical relationship to Pb-MT outcomes. 3) We examined arsenic levels because of theoretical confounding of the Pb Lα peak by arsenic. Arsenic levels in hair samples from residents of a superfund site, (.18 ± .01 ppm, N = 40) and a control town (.18 ± .02 ppm, N = 36) were similar ($p > .10$). From amputated limbs, arsenic was measured in 12 samples of skin without hair, 12 samples of skin and muscle and 12 samples of solubilized tibia. All samples were obtained at the site of LXRF measurements; and all values were equal to or less than 0.001 ppm. We conclude that arsenic does not confound the Pb Lα peak in these cohorts. 4) Improvements in the LXRF instrumentation include a highly efficient spectroscopy system for the once used multichannel analyzer. Software, written by our group, analyzes the Pb Lα peak by the method of cubic splines and autocalibrates the entire system at the beginning of each day's run. There is no spectral overlap; and, hence, no corrections are needed. These data further confirm the utility of LXRF to assess bone Pb in human populations.

65 BIOAVAILABILITY OF A MIXTURE OF METALS

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There are 2000 sites in the U.S. contaminated by coal tar residues from industrial coal gasification. The organic compounds must be identified as well as the inorganic compounds. In the process of coal gasification, As, Cd, Cr, Ni and V are concentrated in the waste. B6C3F1 female mice ingested water containing individual metals for 12 weeks. The mice ingested water

containing a mixture of metals for 24 weeks: As(III), 18 ppm; As(V), 18 ppm; Cd(II), 6 ppm; Cr(VI), 150 ppm; Ni(II), 150 ppm; V(V), 45 ppm. Individual metals, intake water/d, varied from 3.36 ± 0.33 ml/d (As(III)) to 5.21 ± 0.46 ml/d (Ni(II)); mixture of metals in water, intake/d, varied from 3.0 ± 0.3 ml/d to 3.5 ± 0.1 ml/d (S.D.). In the mixture of metals over 12 weeks, the intake was: As(III & V), 118 μg/d; Cd(II), 19.7 μg/d; Cr(VI), 493 μg/d; Ni(II), 493 μg/d; V(V), 14.7 μg/d. In the mixture of metals, As(III & V) increased in the pancreas and kidney, Cd(II) increased in the kidney, Cr(VI) increased in the bone and kidney and V(V) increased in the bone. The data indicate that metals are tissue specific. Supported by the Electric Power Research Institute.

66 MERCURY SPECIATION STUDIES IN SIMULATED TOXIC WASTE SITE SOIL

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As part of a larger project to study speciation of heavy metals in contaminated soil at Superfund toxic waste sites, preliminary data on the speciation of Hg in such soil has been obtained. Soil was collected from selected points on the exterior perimeter of a Superfund site in Texas in order to closely approximate the base soil composition on the site itself. A portion of the soil was spiked with Hg using a standard reference. After being allowed to stand and equilibrate, a soil solution was prepared by extraction with deionized water. The aqueous extract was filtered through a nitrate cellulose membrane with 0.45 micron pore size. Hg was analyzed by Cold Vapor atomic absorption using a VGA-77 Hydride generation attachment to a Varian SpectrAA-20 instrument with stannous chloride as a reducing agent. The initial speciation results indicated that 84.2 % of the Hg was present as water soluble species. The soil residue was collected and prepared for analysis using a microwave digestion sample preparation technique and employing a CEM-2000 MDS Microwave System. Cold Vapor atomic absorption determination indicated that 18.5% of the Hg was retained by the soil as non-water soluble species. The two analyses were found to be in general agreement. The soil solution is currently being further fractionated into other soluble species using a series of exchange resins. The result of this protocol will be a separation into anionic, cationic and neutral species, if present, as well as low polar organic complexes. The methodology involved here will provide a viable strategy for the operational speciation of other heavy metals in contaminated soil. [Supported by ATSDR Grant No. U50/ATU 398948–03]

67 EPIDEMIOLOGIC EVIDENCE FOR THE LONG-TERM RETENTION OF LEAD IN YOUNG CHILDREN

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While a number of studies have shown that lead stored in the skeletal system of adult humans may be released into their blood over biokinetic time scales of several years, there is relatively much less data on long-term retention of lead in human children. Recent longitudinal epidemiology studies allow some indirect estimates of long-term childhood lead retention, particularly intervention studies in which the child's exposure is changed, for example, by soil lead remediation. Estimation of exposure is based on statistical relationships between blood lead and lead concentrations or loadings in the most important external media to which the child is exposed. Changes in exposure have been well correlated with changes in blood lead in groups of lead-exposed children in Boston, MA, and Kellogg, ID, and with observed but unintended changes in studies in Cincinnati, OH, and earlier in Boston. In all of these studies, a substantial part (40 to 60 percent) of the child's blood lead measured 8 to 15 months after the change in exposure cannot be attributed to current lead exposure and appears to reflect primarily historic lead exposure. This suggests that there is a long-term component of lead retention in young children that has a mean residence time of not less than 10 to 12 months, and probably much longer. This is consistent with long-term studies in adult humans and in young non-human primates. Long-term retention should therefore be considered in designing and evaluating risk reduction strategies for childhood lead exposure.

This abstract represents the opinions of the authors and does not necessarily reflect the views of the U.S. Environmental Protection Agency.

68 MERCURY INTOXICATION FOLLOWING A BRIEF INHALATION EXPOSURE

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A 51-yr old Wisconsin resident developed symptoms of acute mercury intoxication while vacationing in Alaska. Her illness was caused by a 15–20 minute exposure to vapors from elemental mercury that was being heated to extract gold from ore she had collected while gold-panning. Three days after the exposure occurred she sought medical treatment in Fairbanks, AK for acute gastrointestinal pain and bloody diarrhea. At that time her urine mercury level was 554 ug/1000 mL/24 hr (normal < 20). When evaluated in Wisconsin 11 days after exposure, she complained of joint pain and had a blood mercury level of 0.2 ug/mL (normal < 0.005). Renal and liver function were normal. By day 28, her blood mercury level had declined to 0.038 ug/mL. On day 51 she was asymptomatic and laboratory tests revealed a blood mercury level of 0.015 ug/mL and a urine mercury level of 262 ug/2520 mL/24 hrs (104 ug/L). Analysis of these data yielded a blood compartment half-life of ~10 days and a total body burden of at least 20 mg of mercury. Assuming a respiratory rate of 1 cu.m/hr and an absorption efficiency of 80%, a mercury concentration of ~100 mg/cu.m air was estimated.

69 ANALYSIS OF EDTA-CHELATABLE PROTEINS FROM DNA-PROTEIN CROSSLINKS INDUCED BY THE CARCINOGENIC CHROMIUM(VI) IN CULTURED INTACT HUMAN CELLS

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DNA-protein crosslinks were induced in intact human leukemic T-lymphocyte MOLT4 cells or isolated nuclei by treatment with potassium chromate, chromium(III) chloride hexahydrate or x-ray. The proteins complexed to DNA were analyzed by two-dimensional SDS-polyacrylamide gel electrophoresis (PAGE). A group of identical nonhistone proteins were found to crosslink to DNA by potassium chromate, chromium(III) chloride hexahydrate, and x-ray except that a 51 kD basic protein was additionally complexed to DNA when either potassium chromate or chromium(III) chloride hexahydrate was the crosslinking agent. Treatment of chromate-induced DNA-protein crosslinks with EDTA or thiourea followed by ultracentifugation dissociated the major proteins from the complexes indicating that those proteins were crosslinked by direct participation of an EDTA-chelatable form of chromate such as Cr(III) through sulfur containing amino acid residues. The 51 kD protein was not seen in the post-EDTA pellet but was present in the post-thiourea pellet, indicating that it was also crosslinked by Cr(III) through non-sulfur-containing amino acids. The majority of proteins that were crosslinked to DNA were resistant to EDTA and thiourea treatment, and were also crosslinked by x-ray, indicating the involvement of oxidative mechanisms in the process. These results suggest that the chromate-induced DNA-protein crosslinks are formed, at least in part, by the generation of active oxygen species during the intracellular chromate reduction.

70 ACQUISITION OF Pb BY YOUNG CHILDREN

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We have followed the blood Pb concentrations and isotope ratios in 40 Omaha children of various ages for periods ranging from 2 to 5 years. At birth and at 6 months there is on average no change in blood Pb concentration (1.0 ± 0.5 *vs.* 1.1 ± 0.5 μg/dL), but over the next 6 to 12 months a 4-fold rise to 4 ± 2 μg/dL occurs. Thereafter the blood Pb concentrations show no significant change. Important exceptions to this general picture occur. Blood Pb concentrations of some children consuming 2 to 4 μg Pb/day in infant formula decline by 25 to 50 percent during the first 6 months of life, which implies that their dietary absorption of Pb is less than 10 per cent, a figure far lower than current estimates. The Pb isotope ratios in their urine, which move away from those of their diet over the same period, also indicate that the diet contributes insignificantly to their blood Pb. Some children have been briefly exposed to Pb of unusual isotope ratio at about 1 year, raising their blood Pb to above 10 μg/dL. This Pb is eliminated with a half life of 30 months and may be detectable with isotopes as long as 4 years after exposure. At the other extreme are a few who born with blood Pb less than

1 μg/dL have remained at that value for two years. Supported by NIEHS grant #ES04762 to C R Angle.

71 A NON HUMAN PRIMATE MODEL FOR LEAD KINETICS IN GERIATRIC HUMAN POPULATIONS

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The purpose of the investigation was to study lead (Pb) kinetics in a non human primate model of aging using K XRF measurements of Pb in bone. Rhesus monkeys (*Macaca mulatta*) were administered Pb acetate daily in drinking water for six years during adulthood; controls were given no Pb. Pb content in tibia was measured using ^{109}Cd K XRF techniques at 28 ± 2 yrs of age and again approximately 10 months later. Pb intake ended 10 years prior to the first bone measurement. Bone Pb content was significantly elevated for the Pb-treated monkeys and there were no significant changes over the repeated measurements. The accumulation rate of Pb into tibia was similar to that measured in humans, 0.1 mcg Pb/(g bone mineral)/mcg/dl yr) in monkey and 0.05 to 0.1 in human. A half-life of Pb in a single bone compartment for the Pb treated monkeys was calculated to be 3.0 ± 1.0 years. Endogenous Pb exposure from bone was low at the time of bone Pb measurements but may have been higher from cortical than from trabecular bone. The rhesus monkey appears to be an excellent model of human bone Pb metabolism studies needed for further understanding of Pb kinetics in geriatric populations. Supported by EPA CR-817425 and CR-817156.

72 TRENDS IN BLOOD LEAD VALUES IN UNCHELATED CHILDREN WITH MODERATE LEAD POISONING

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This study determined contributions of environmental Pb exposure and Fe status to changes in BPb levels over six months in moderately Pb poisoned children (initial BPb: 25–55 μg/dL). Chelation therapy was not administered: all children had negative lead mobilization tests (Pb-MT) indicating limited response to $CaNa_2EDTA$. Children received these interventions: three home visits to assess lead paint hazards; notification of the D.O.H. to remediate lead hazards; educational information about the routes of lead exposure, its toxicity and treatments; Fe therapy for children with ferritin levels < 16 μg/dL; and 10 clinic visits over the six month period to reassess clinical status, reinforce educational information and obtain BPb levels. Other than a single dose on the day of a Pb-MT, no child received chelation. To quantify lead paint hazards, a visual rating of the surfaces (intact to peeling) was combined with x-ray fluorescence measurements. The sum of these assessments was termed the home environmental score (HES). Data were analyzed from 79 children. BPb levels declined by 27% over the six months. HES was correlated with BPb at enrollment but neither the initial nor later HES measurements predicted BPb. The HES was highest at enrollment and declined by 50% and 75% at the second nad third home visits, respectively. Only a minority of children (20%) achieved a HES of 0, indicating no lead paint hazards. Despite some ongoing Pb exposure, a parallel fall in BPb levels was observed in subgroups of children with initially low or high HES. Fe status did not account for the change in BPb levels: Fe deficient and sufficient children had comparable rates of decline in BPb concentrations. These indicate that: 1) the HES is quantifiably related to BPb levels; 2) this correlation is significant only prior to intervention, and, 3) BPb levels decline in moderately lead poisoned children after they are enrolled in a comprehensive intervention program, even in the absence of chelation therapy and in the presence of some ongoing lead paint exposure and Fe deficiency.

73 PHARMACOKINETICS OF DRINKING WATER EXPOSURE TO SELECTED CHROMIUM (III AND VI) COMPOUNDS IN HUMAN VOLUNTEERS

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This study examines chromium uptake and excretion following ingestion of a single oral dose of three different chromium compounds in water ($CrCl_3$, K_2CrO_4 reduced in orange juice, and K_2CrO_4). Adult volunteers ingested a single dose of 5 mg chromium in a 0.5 liter volume. Blood and urine samples collected for up to two weeks following the dose were analyzed for total chromium. Plasma and red blood cell (RBC) chromium concentrations were transiently elevated for all three chromium compounds within 8 hours after the dose. Total urinary chromium excretion within 4 days after the dose was less than 1% of the dose for $CrCl_3$ and K_2CrO_4 reduced in orange juice, but was considerably higher for the same dose of K_2CrO_4 in water. Despite the greater systemic chromium uptake and excretion following ingestion of K_2CrO_4 in water, no sustained elevation of chromium was observed in red blood cells — a marker for systemic uptake of the hexavalent form. The data suggest that ingestion of trivalent chromium as $CrCl_3$ or as a probable organic complex in orange juice leads to low level systemic uptake, indicated by transient elevations in RBC chromium content and slightly increased urinary chromium excretion. The magnitude of total chromium uptake is increased when K_2CrO_4 is administered in water, but the absence of any sustained elevation of RBC chromium levels indicates reduction intragastrically followed by systemic absorption of the trivalent form.

74 DERMAL UPTAKE OF HEXAVALENT CHROMIUM IN HUMAN VOLUNTEERS MEASURES OF SYSTEMIC UPTAKE FROM IMMERSION IN WATER AT 22 PPM

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This study examines the systemic uptake of chromium in four human volunteers following 3 hours of contact with water containing hexavalent chromium [Cr(VI)] at a concentration of 22 ppm (mg/L). Volunteers were immersed below the shoulders (about 13,000 cm^2) in the water at 91 ± 2.5° F. On the day prior to the experiment and for six days afterwards, samples of urine, plasma, and red blood cells were collected and analyzed for total chromium. Red blood cell chromium concentrations were used as a specific biomarker for systemic uptake of hexavalent chromium. No sustained elevation of chromium concentrations was observed in red blood cells of the volunteers tested; thus, no appreciable Cr(VI) was systemically absorbed. Small increases were observed in the concentration of chromium in urine within 48 hours of exposure, indicating some Cr(III) may have penetrated the skin at a rate of about 3.5 x 10^{-5} to 5.2 x 10^{-4} μg/cm^2-hr. In short, dermal exposure of humans for 3 hours at 22 ppm Cr(VI) did not result in systemic uptake of measurable amounts of Cr(VI), but a very small quantity of chromium may have penetrated the skin where it was subsequently reduced to Cr(III) before systemic uptake and distribution.

75 HANDDUST LEAD LOADING (μg/m^2) AS AN IMPROVED PREDICTOR OF CHILDHOOD BLOOD LEAD (PbB) IN LONGITUDINAL STUDIES

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Handwipe lead (PbHW) is often evaluated as an exposure index reflecting the exchange between soil and dust lead and childhood blood lead (PbB). Since PbHW, usually obtained by 2 wipes of both sides of both hands, increases with surface area (SA), PbHW/5% body SA (PbHWSA μg/m^2) may better reflect Pb loading in longitudinal studies of growing children. Circulating PbB (8% wt kg x PbB) similarly reflects the effect of growth on body burden: while PbB declines with age, PbBcirc may be stable or increase. In the 12 month Omaha Study of 21 urban children, 2–3 y.o., PbHW was 5.6 μg ± 4.5 (SD); PbHWSA was 3.4 μg/m^2 ± 2.9 (n = 244); PbB was 6.4 ± 3.1 μg/dL; PbBcirc was 73.2 ± 35.1 μg (n = 82). Although the prediction of PbB by PbHW and age (ANOVA correlated for repeat measures) was not statistically significant, PbBcirc was significantly predicted by PbHWSA: p = .011, SEE 0.6. Physiologic models may provide more valid correlates of environmental exposure than the use of age as a predictive covariable in multiple regression analyses, particularly in longitudinal studies of environmental lead and the body burden of childhood lead. Supported by NIEHS #ES04762.

76 TESTING FOR DNA-PROTEIN CROSSLINKING AFTER DRINKING WATER EXPOSURE TO CHROMIUM (III AND VI) IN HUMAN VOLUNTEERS

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Increased DNA-protein crosslinking (DPX) in circulating lymphocytes has been proposed as a potential biomarker for exposure and potential genotoxic

damage caused by inhalation of certain reactive chemicals, such as hexavalent chromium. This study was designed to determine whether ingestion of a single dose of chromate alone [Cr(VI)] or chromate fully reduced to Cr(III) with orange juice causes an increase in DPX of circulating lymphocytes in humans. Five adult male volunteers ingested a dose of 5 mg chromium in a 0.5 liter volume of water, and blood samples were collected at 0, 30, 60, 120, 180, and 240 minutes afterwards for analysis of lymphocyte DPX. Blood and urine samples were also collected for up to two weeks following the dose to examine the pattern of uptake and excretion of chromium. The results demonstrated that no significant change in DPX was observed following either Cr(VI) or Cr(III) ingestion, even though blood and urine chromium measurements indicated systemic uptake and urinary excretion of a substantial fraction of the ingested chromium within 4 days after the dose. Since Cr(III) does not possess DPX-inducing properties while Cr(VI) does, these results suggest that the Cr(VI) was reduced to Cr(III) intragastrically prior to absorption.

77 REDUCTION KINETICS OF HEXAVALENT CHROMIUM IN HUMAN BLOOD

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Available literature suggests that both plasma and red blood cells are capable of reducing hexavalent chromium to the less reactive trivalent form. However, the rate of reduction and the maximum capacity for reduction in blood have not been well characterized. To evaluate these parameters, fresh human blood stabilized with EDTA was separated into red blood cell and plasma pools. The plasma was spiked with Cr(VI) at concentrations ranging from 0.2 to 10.0 ppm. The blood was then reconstituted and incubated at body temperature. Timed samples were removed for total chromium and hexavalent chromium analyses in both red blood cells and plasma. Cr(VI) spiked into plasma alone was stable for several hours at 0.003 – 0.025 ppm, indicating a lack of appreciable reductive capacity in isolated plasma. Red blood cells exhibited a large capacity to reduce Cr(VI) and accumulate chromium (perhaps greater than 15 ppm). The rate of Cr(VI) reduction in whole blood is extremely rapid and complete at concentrations below 2.0 ppm, but plasma compartment reduction processes apparently are overwhelmed at a concentration of 10 ppm Cr(VI). The studies indicate that the blood provides a significant buffer or barrier to prevent the distribution of Cr(VI) in the body via chromium reduction in plasma (in the whole blood matrix) and rapid uptake and reduction inside red blood cells.

78 BIOAVAILABILITY OF SOIL-BORNE LEAD IN ADULTS, BY STABLE ISOTOPE DILUTION

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Using stable isotope dilution, we determined the bioavailability of soil-borne lead in human adult volunteers. Soil from a residential yard at a mining-impacted federal Superfund site, which had negligible amounts of other priority pollutants, was dried and screened through a 250 μm mesh sieve. The < 250 μm fraction, which likely represents that ingested via hand-to-mouth activity, was then sterilized by exposure to radiation. Ten replicate samples yielded a mean (SD) soil lead concentration of 2924 ± 36 ppm, and a mean $^{206}Pb/^{207}Pb$ ratio of 1.1083 ± 0.0002, indicating remarkable soil homogeneity. Six adults with $^{206}Pb/^{207}Pb$ ratios of > 1.190 were admitted to the Clinical Research Center and fasted overnight prior to dosing with 250 μg Pb/70 kg body wt (i.e., 85.4 mg soil/70 kg) in a gelatin capsule. Blood for Pb and $^{206}Pb/^{207}Pb$ ratios was obtained at 14 time points through 30 hours. Isotopic analyses from two subjects have been completed and indicate that 35.6% and 22.6%, respectively, of the administered dose was absorbed. (Supported by EPA Agreement CR 822793 and EPA Region II.)

79 DISPOSITION AND METABOLISM OF OCTAMETHYL-CYCLOTETRASILOXANE (D_4) IN F-344 RATS: EFFECT OF CLASSICAL INDUCING AGENTS

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Previous studies in our laboratory have shown repeated inhalation exposure to high levels of octamethylcyclotetrasiloxane (D_4) causes liver enlargement with significant induction of P450 2B1 and a slight induction of P450 1A1 in rats. In order to evaluate the potential role of induction on the metabolism of D_4, female rats were pretreated with either phenobarbital (PB) (80 mg/kg, i.p.), 3-methylcholanthrene (3-MC) (30 mg/kg, i.p.), or vehicle once a day for four consecutive days. On the following day, rats were administered a single dose of ^4C-D_4 (70 mg/kg, i.v.). Urine, feces and expired air were collected over the next 72 hr. PB treated rats excreted ≈ 50% of the administered dose in the urine, while 3-MC and control rats excreted ≈20% over the same 72 hr period. However, only ≈5% of the dose was excreted as expired volatiles in PB treated rats, while 3-MC and control rats excreted ≈30% over the 72 hr period. At 72 hr following administration of D_4, ≈30% of the dose remained in the control and 3-MC pretreated carcass compared with ≈5% in the PB treated rats. HPLC analysis of urine revealed no qualitative change in PB or 3-MC pretreated animals when compared to controls. A similar profile of at least 6 metabolites of D_4 and no parent compound was detected in all samples. However, pretreatment with PB did increase the formation of a novel polar metabolite, methylsilane triol at later time points when compared to controls. The results of this study indicate PB pretreatment increased the excretion rate and the metabolism of D_4 which lead to an increased formation of methylsilane triol as a urinary metabolite. These results suggest P450 2B1 is one of the potential isozymes involved in the metabolism of D_4.

80 EVALUATION OF UDP-GLUCURONOSYL TRANSFERASE, α-GLUTATHIONE-S-TRANSFERASE, & EPOXIDE HYDROLASE IN RAT LIVER FOLLOWING REPEATED INHALATION EXPOSURES TO OCTAMETHYLCYCLOTETRASILOXANE (D4)

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Previous studies in this laboratory have shown that D4 induces at least two classes of P450 isozymes (1A1 and 2B1). *In vivo* metabolism studies suggest that D4 is reduced to low molecular weight, water soluble components. However, the role of glucuronide and glutathione conjugation in the metabolism of D4 has not been examined. The purpose of the present study was to evaluate changes in hepatic α-Glutathione-S-transferase (α-GST), UDP-Glucuronosyltransferase (UDPGT), and Epoxide Hydrolase (EH) following exposure to D4. Male and female Fischer 344 rats were exposed to 0, 70, and 700 ppm D4 in whole body inhalation chambers for 6 hr/day, 5 days/week, for 28 days. Animals were sacrificed on day 28 and hepatic microsomes and cytosol were prepared by differential centrifugation. Changes in microsomal UDPGT activity were assessed using p-nitrophenol as substrate. No significant differences in UDPGT activity were observed in control versus treated groups. In addition, D4 exposure did not change α-GST levels in cytosol, as determined by ELISA. The effect of D4 on microsomal EH was evaluated by measuring changes in EH mRNA levels. Analysis of EH mRNA showed a significant increase in female 70 and 700 ppm groups (~ 3–4 fold) compared to controls. No significant differences between control and treated groups were observed in males. Whether or not EH is directly involved with the breakdown of D4 or induced by indirect mechanisms is unknown. Overall, these findings are in agreement with *in vivo* D4 metabolism studies and suggest that phase II conjugation reactions may not play a major role in the metabolism of D4.

81 INDUCTION OF HEPATIC P450 1A1 AND 2B1 FOLLOWING REPEATED INHALATION EXPOSURE TO DECAMETHYL-CYCLOPENTASILOXANE (D5)

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D5 is one member of a group of cyclic siloxanes which are used in a variety of commercial and industrial applications because of their physical and functional performance. Previous studies from this laboratory have shown that exposure to octamethyl-cyclotetrasiloxane (D4), increases liver size and induces P450 1A1 and 2B1 in a dose-dependent manner. The purpose of the present study was to determine if exposure to D5 by inhalation would produce a similar increase in hepatic P450 1A1 and 2B1 isoenzymes. Female Fischer rats were exposed to 0 and 160 ppm D5 in whole body inhalation chambers for 6 hr/day, 7 days/week, for 28 days. Following exposure, liver was removed and microsomes prepared by differential centrifugation. In D5 animals liver to body weight ratios increased 17% over controls. After 14 days post-

exposure, liver size returned to normal. P450 1A1 activity was assessed using ethoxyresorufin-O-deethylation (EROD) and P450 2B1 activity was assessed using pentoxyresorufin-O-depentylation (PROD). At the end of the exposure period EROD activity was increased approximately 2-fold, while (PROD) increased approximately 4-fold over controls ($p<0.05$). A comparable increase in 2B1 protein was measured using an ELISA. The suicide inhibitor 9-ethynylphenanthrene, which is selective for the P450 2B1 subfamily, reduced PROD activity by 42% and 75% at 0.1μM and 1.0μM respectively. These data suggest that like D4, D5 produces transient hepatomegaly and increases the activity of P450 1A1 and 2B1 isoenzymes. Thus, these studies provide insight into the mechanism(s) by which cyclic siloxanes are metabolized *in vivo*.

82 DISPOSITION AND METABOLISM OF OCTAMETHYLCYCLOTETRASILOXANE (D_4) IN MALE AND FEMALE RATS FOLLOWING REPEATED NOSE-ONLY VAPOR INHALATION EXPOSURE

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To further elucidate the disposition of radioactivity observed after a single 6 hr nose-only vapor inhalation exposure to ^{14}C-D_4, male and female Fischer 344 rats were exposed on a repeated basis. Animals were exposed to target concentrations of 700 ppm D_4 vapor for 14 consecutive days followed by a single exposure to ^{14}C-D_4 on day 15 via a nose-only inhalation chamber. Immediately following exposure, one group of animals was solubilized and total ^{14}C body burden was determined. Excreta animals were placed in individual metabolism cages for 168 hr post ^{14}C-D_4 exposure for collection of urine, feces and expired air. Additional animals were euthanized at predetermined time points for blood and tissue collection to evaluate effects on plasma and tissue kinetics. Radioactivity content was measured in all samples. Approximately 5% of the total radioactivity the animals were exposed to was retained in the body burden animals at the end of exposure. Of the recovered radioactivity in the excreta animals, the majority (<90%) was eliminated via the urine, expired air and feces. Approximately 38% was excreted in the urine, 36% in expired volatiles, 18% in the feces and 4.5% as CO_2 in both sexes. Differences seen in the excretion profile between the males and females following the single exposure were not reproduced in this study. Urinary metabolite profiles are being determined for both the single and repeat exposure studies to evaluate if the metabolic pathway may have been altered following the repeated exposure to D_4 vapor.

83 ISOLATION OF SILOXANE METABOLITES: AN EFFICIENT EXTRACTION

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Disposition and metabolism of octamethylcyclotetrasiloxane (D_4) in humans and rodents are currently being investigated. In order to identify and quantitate D_4 and potential metabolites from various biological matrices, an efficient extraction procedure into a suitable solvent is critical. A number of common organic solvents such as hexane, dichloromethane, and acetonitrile were evaluated for their ability to extract D_4 and potential metabolites from a variety of biological samples such as blood, plasma, urine, feces and selected tissues. Samples were either spiked with ^{14}C-D_4 or obtained from animals administered ^{14}C-D_4 via various routes of exposure. Extraction efficiency was poor for most of the solvents due to the insolubility of either D_4 or the metabolites. An extremely efficient extraction procedure has been developed to recover D_4 and its potential degradation products using tetrahydrofuran (THF) as the solvent medium. THF consistently demonstrated an extraction efficiency of greater than 90% with most of the biological matrices. Plasma and blood spiked with ^{14}C-D_4 showed an extraction efficiency of > 90% following 3 extractions with THF. Blood, plasma and urine obtained from animals administered ^{14}C-D_4 (i.v.) also showed a > 90% recovery of radioactivity following the THF extraction procedure. In addition, HPLC profiles obtained before and after the extraction showed identical profiles. These results indicate that THF is a suitable solvent for the extraction of D_4 and its potential 2metabolites from a variety of biological matrices.

84 IDENTIFYING AND SOLVING AN INDOOR AIR PROBLEM IN AN ELEMENTARY SCHOOL

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An elementary school was closed in Sept 1994 following reports of headaches, nausea, and upper respiratory and eye irritation by staff and students after exposure to an unusual "vinyl-like" odor in the classrooms. A symptom survey of staff and students indicated that there was a significant increase in headache prevalence during the period they were exposed to the odor. Preliminary air sampling for volatile organic compounds inside the classrooms showed a similar chemical profile to those obtained from the attic and from a chamber study of a small sample of the school's roof. Indoor odors also suggested off-gassing from the deteriorating roof.

An extensive investigation was undertaken, involving sampling for more than 135 chemicals, as well as materials from the ducts and airborne fungal spores samples from classrooms. Several mitigation measures were undertaken, such as sealing all wood stud framing and roof vent riser cracks, and painting the roof with a reflective white paint to reduce the temperature. These efforts were successful in removing the odor from the classrooms, but the precise source and chemicals causing the odor could not be identified. Evaluation of air sampling results indicated that the chemicals detected in the classrooms, either before or after the mitigation efforts, were below concentrations likely to cause any adverse long-term health effects.

Nevertheless, the school's roof was replaced, largely to allay parental concerns about potential health effects.

85 IMMUNOLOGICAL EFFECTS OF RESPIRATORY EXPOSURE TO SILOXANE IN HUMANS

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Humans are exposed to silicones in a large number of commercial consumer products. Some of these silicones, including octamethylcyclotetrasiloxane (D_4), are volatile and/or are used as sprays; therefore, there is a potential for exposure via the respiratory route. Controlled human exposure is essential for understanding the pharmacokinetics of D_4 and other volatile siloxanes and these exposure provide an opportunity for investigation of the immunological effects of these same compounds. The effects of inhalation on the immune system were studied in a double-blind, placebo-controlled, crossover study. Twelve healthy subjects 25–47 years old inhaled D_4 at 10 ppm or air for one hour. Blood was obtained for immunological studies just prior to exposure, immediately post exposure, and 6 and 24 hrs after exposure. Assays included: Complete blood count with differential, c-reactive protein, erythrocyte sedimentation rate, serum IL-6, and flow cytofluorometry studies of lymphocyte subsets. In addition, peripheral blood mononuclear cells were isolated and assayed for proliferation in response to phytohemagglutinin and alloantigens, and ability to secrete IL-2, γ interferon, and TNF_α. Natural Killer cell cytotoxicity was measured immediately and 24 hrs after exposure. A slight decrease was found in PHA-induced proliferation and other immunological parameters immediately post exposure but similar decreases were seen with both the air and D_4 exposures. In these initial studies we found no immunological effect of D_4 inhalation in humans.

86 ADVERSE PULMONARY EFFECTS OF MACHINING FLUID AEROSOLS

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This laboratory has previously demonstrated that significant changes in pulmonary function and increases in biochemical and cellular markers of lung injury occur after a single 3 hr exposure to as little as 10 mg/m^3 used machining fluid aerosols, while unused machining fluid aerosols produce minimal effects even at 100 mg/m^3. Although only one machining fluid type was tested (soluble), those results suggested that contaminants such as endotoxin, which occur during storage and handling of the machining fluids, could play a significant role in producing the adverse respiratory effects observed in workers encountering machining fluid aerosols. The present study has expanded our initial findings and examined the relative toxicity of 3 of the 4 major classes of machining fluids (soluble, semi-synthetic, and synthetic) as well as the contribution of various components/factors of the fluids. Significant differences in toxicity were observed among the adverse effects produced by aerosols of the unused machining fluids. The study also found that for

all 3 machining fluids, greater toxicity was observed in guinea pigs exposed to the used (i.e., contaminated) compared to the unused machining fluids. A low-effect level of 5 mg/m^3 was observed for changes in biochemical and cellular markers of injury after a single 3 hour exposure to aerosols of used machining fluids. In addition, adjusting the unused semi-synthetic machining fluid to pH 7 and 290 mOsm, decreased its toxicity. Together, these findings strongly suggest that multiple factors contribute to the adverse respiratory effects associated with occupational exposure to machining fluid aerosols.

87 TOXICOLOGICAL AND HUMORAL IMMUNITY ASSESSMENT IN RATS EXPOSED FOR 28 DAYS TO DECAMETHYLCYCLOPENTASILOXANE (D5) VAPOR

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D5 is a low molecular weight cyclic siloxane used in the personal and household care industries. The objective of the present study was to assess potential toxic and immunotoxic consequences of inhalation exposure to D5. Male and female F344 rats (25/group) were exposed (whole body) to 0, 10, 25, 75, or 160 ppm D5 6 hr/d, 7 d/wk for 28 d. Clinical signs, body weights (BW), and food consumption (FC) were recorded. On day 29, 10 rats/group/sex were euthanized and a complete necropsy performed. Following a 14 d recovery, 5 rats/sex/group were necropsied. Body and organ wts were obtained and a complete set of tissues was taken for histopathology. Samples were also collected for serum chemistry, hematology, and urinalysis. Immunotoxicology rats (10/sex/group) were immunized with sRBC 96 hr prior to euthanasia and CYP was administered i.p. to positive controls on days 24–28. The anti-sRBC AFC response was evaluated in a standard plaque assay. D5 exposure caused no adverse effects on BW, FC, or urinalysis parameters. Significant decreases were noted in RBC count, hematocrit, and hemoglobin in females (2–8%, 160 ppm) which were not present in recovery rats. SAP was observed to be significantly decreased in females (17%, 160 ppm) at terminal and recovery sacrifice. A significant increase in liver:BW ratio was observed in females (13%, 160 ppm) which was not present in recovery rats. In males, significant increases in liver:BW (5%) and thymus:BW (14%) ratios were also noted at the high dose at terminal sacrifice and were not present at recovery. At recovery only, a significant increase in spleen:BW ratios (14 and 17%, 25 and 160 ppm) was noted. D5 inhalation exposure did not alter humoral immunity and caused only minor, transient changes in hematological, serum chemistry, and organ weight values.

88 GROWTH OF A PYROTECHNICALLY GENERATED AEROSOL (SFE) AS A FUNCTION OF RELATIVE HUMIDITY

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Varying rates of particle growth of SFE aerosols (up to 4 μm in 30 minutes) have been observed for two nominal concentrations (50 and 80 g/m^3) at relative humidity ranging from 30 to 100%. Particle growth was attributed to coagulation and hygroscopicity. Hygroscopic growth of aerosol particles has been shown to be an important factor in particle deposition. A series of experiments were conducted to determine particle growth rates at various known relative humidities and at lower concentrations to minimize the coagulation effect. An estimate of the relative particle growth as a result of atmospheric moisture in the initial observations was made. These experiments provide a basis for estimation of the additional hygroscopic growth to equilibrium size subsequent to inhalation and, therefore, changes in deposition. Observed particle growth attributed to hygroscopicity was compared to predicted growth as a function of relative humidity.

89 TOXICOLOGICAL EVALUATION OF EXPOSURE TO TWO FORMULATIONS OF A PYROTECHNICALLY -GENERATED AEROSOL

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Fischer 344 rats (250–300 g) were exposed to the by-products of two formulations of SFE Formulation A, a pyrotechnically-generated aerosol fire extinguishant. Exposures levels ranged from 50 g/m^3 (nominal concentration) to 240 g/m^3. The length of exposure was either 15 or 60 minutes under static conditions. A 700 L whole-body inhalation chamber was used for the exposures and consisted of a supply/exhaust system, aerosol generator and exhaust scrubber. The chamber atmosphere underwent aerosol [size (MMAD), distribution (σg) and concentration] and gas [CO, CO_2 and O_2] analysis. Clinical observations were taken throughout the exposure. Animals were euthanized 1-hr post-exposure and underwent histopathological examination and blood gas analysis. The difference in formulation caused dramatic results in survivability. The first formulation produced levels of carbon monoxide very close to lethal concentrations, while the second formulation showed little if any production. The lack of carbon monoxide production during pyrolyzation is the key difference in the survivability and toxicity of the two formulations.

90 THE EFFECTS OF OCTAMETHYCYCLOTETRASILOXANE (D_4) IN A 90 DAY NOSE-ONLY VAPOR INHALATION STUDY IN RATS

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D_4, a cyclosiloxane, has many applications in consumer, personal care and industrial products. Groups of 20 male and 20 female Fischer 344 rats (control and high dose 30/sex) were exposed nose-only to 0, 0.3, 1.2, 5.0 or 12.0 mg of D_4/l, 6 hours/day, 5days/week for three months. Following the treatment period, 10/sex in the control and high dose group were allowed a 28 day recovery period prior to sacrifice. Body weights, food consumption and clinical signs were recorded. 24 hours following the last exposure (or on day 28 following the last exposure for recovery groups), animals were sacrificed and blood and urine obtained for clinical chemistry, hematology and urinalysis. A complete necropsy was performed on all animals and tissues obtained for histopathology. Selected organ weights were also obtained. With the exception of the high dose group, food consumption, body weights and body weight gains were not affected by D_4 exposure. In the high dose group, there was a significant ($p \leq 0.05$) inhibition in overall body weight gain. With the exception of a moderate increase in gamma-glutamyltransferase activity which returned to control values following recovery, no significant adverse effects were noted in clinical signs, hematology, clinical chemistry or urinalysis parameters evaluated. A dose dependent statistically significant increase in absolute and relative liver weights was seen in treated animals however, following recovery, no significant differences were noted and microscopically no alterations were noted. Histopathologic findings were generally limited to the two highest doses and involved minimal goblet cell proliferation in the nasopharyngeal duct, minor influx of alveolar macrophages in the lungs, slight ovarian atrophy and vaginal mucification (high dose only). During the recovery period essentially all of the histological alterations were reversible.

91 ESTIMATING THE MAXIMUM TOLERATED DOSE (MTD) FOR A FIBER CHRONIC INHALATION STUDY IN RODENTS

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Short-term toxicity and lung clearance were assessed in rats exposed by inhalation to size-selected fibrous glass (FG) for 13 weeks. Results from this study and from a recent FG chronic inhalation study are presented here as guidelines for the selection of a maximum tolerated dose (MTD) for chronic inhalation studies of fibers. Fisher 344 rats were exposed using nose-only inhalation chambers, 6 hrs/day, 5 days/week, for 13 weeks to one of 5 concentrations (3, 16, 30, 45 or 60 mg/m^3) of FG test fiber or to filtered air (control), then held for an additional 10 weeks of recovery. Test fiber was size-selected from glass wool having a chemical composition representative of building insulation. Rats were sacrificed at 7, 13, 19 and 23 weeks after the onset of exposure to evaluate: pulmonary pathology; lung epithelium cell proliferation; lung fiber burden; and lung lavage cells and chemistry. The effect of fiber inhalation on lung clearance of innocuous microspheres was also evaluated as follows: At 13 wks, 6 animals/group were exposed to ^{85}Sr-labeled 3.0 μm latex microspheres by intratracheal inhalation and then monitored for whole-body radioactivity during the 10-week recovery period. Data from the short-term study support the choice of 30 mg/m^3 as the MTD for the previous chronic FG study and also provide indicators of long-term lung toxicity and functional impairment that can be used to estimate the MTD for future chronic fiber inhalation studies.

92 ACUTE INHALATION TOXICITY OF CARBONYL SULFIDE

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Carbonyl sulfide (COS), present in several commodity fuels including propane and synthesis gas, is rapidly metabolized to H_2S in the body. The purpose of this research was to determine the effects of exposure concentration and time on the toxicity of acutely inhaled COS in F344 rats and to compare the toxic effects with those of H_2S. Groups of male and female rats were exposed by nose-only inhalation to 250 – 1400 ppm COS for up to 4 hr and observed for up to 14 days. No lethality was observed below 500 ppm; the median lethal concentration was estimated at 590 ppm. Time to death was 30 – 60 min from the start of the exposure at 1400 ppm, 2 – 3 hr at 1000 ppm, 3 – 4 hr at 750 ppm, and > 4 hr at 590 ppm. Exposure to the higher concentrations caused a phase of excitation followed by a period of depression in which heart rate and body temperature were reduced. Gross pathological changes were minimal in rats inhaling 1400 ppm; most body organs, including lung, liver, kidney and GI tract were severely congested in rats inhaling 750 – 1000 ppm. Rats surviving exposure to > 500 ppm COS had motor impairment consistent with damage to the vestibular-cerebellarstriatal pathways. Preliminary histopathology showed swelling of myelin sheaths in the corpus callosum, cerebellum, and pyramidal tract. Partial-to-complete resolution of the gross pathological and behavioral changes occurred during the 14-day post exposure observation period. [Research sponsored by the Office of Environmental Restoration, U.S. Department of Energy under Contract No. DE-AC04-76EV01013.]

93 *IN VITRO* EFFECTS OF DUSTS ON PULMONARY CELLS USING MACROPHAGE AND EPITHELIAL CELLS IN CO-CULTURE

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The current studies were designed to evaluate two pulmonary cell-types, i.e., rat lung epithelial (L2) cells and rat alveolar macrophages (AM) for *in vitro* studies with particulates and to correlate the results of *in vitro* studies with data generated from inhalation studies in rats using the same particle-types. L2 cells, AMs, or both cell-types in combination (i.e., co-cultured) were incubated at various concentrations with zinc oxide (ZnO) particles or titanium dioxide (TiO_2) particles. Cytotoxicity was assessed using the MTT assay, as well as by measuring for lactate dehydrogenase activity in the culture fluid. The data obtained form the *in vitro* assays were then compared to the effects measured following 6 hour or 3-day exposures to each of these dusts.
The results of *in vitro* studies demonstrated that ZnO particles were extremely toxic to L2 cells but had little effect on AMs. When the two cell-types were co-cultured, AMs could not protect the toxic effects of ZnO to L2 cells. In contrast, TiO_2 particles at 3 doses had no significant effect upon either L2 cells or AMs. Following a 1 or 3-day inhalation exposure to ZnO particles at concentrations ranging from 10 to 100 mg/m^3 or to TiO_2 particles at 100 or 250 mg/m3, rats were sacrificed at sequential postexposure time periods and their lungs lavaged. Inhalation of ZnO caused a potent but short-lived pulmonary inflammatory response, suggesting rapid clearance. Three-day exposures to TiO_2 particles at 100 mg/m^3 produced a short-lived neutrophilic response without corresponding BAL parameters of inflammation. However, 3-day exposures to TiO_2 at overload concentrations (250 mg/m^3) produced transient inflammatory responses in the absence of continuing exposure. The results of this study demonstrate the need for better validation in carrying out *in vitro* studies with particles and fibers.

94 COMPARISON OF SENSORY AND PULMONARY IRRITATING EFFECTS OF AMINES FOUND IN METALWORKING FLUIDS (MWF)

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In this study, a mouse bioassay was used to assess the sensory and pulmonary irritation of amines found in MWF: 3 ethanolamines (mono-, di-, tri-) and 3 isopropanolamines (mono-, di-, tri-). Each amine was aerosolized for 3 hours, during which time the respiratory pattern and frequency (*f*) of mice were monitored. Sensory irritation (S) occurred rapidly upon exposure to each amine. With the isopropanol-amines, pulmonary irritation (P) also occurred later in the exposures. As a result of S and/or P, *f* decreased. Using *f*, a concentration-response relationship was developed for each amine. From such relationships, the concentration producing a 50% decrease in *f* due to S or P was determined ($RD_{50}S$ or $RD_{50}P$). $RD_{50}S$ values for the ethanolamines ranged from 500–1500 mg/m^3 while $RD_{50}P$ values for the isopropanolamines ranged from 400–4,000 mg/m^3. Within the series of ethanolamines, di- and tri- were similar in (S) potency to one another and both were more potent than mono-. Within the series of isopropanolamines, mono- and tri- were similar in (P) potency to one another and both were more potent than di-. These toxicological data may be helpful in selecting amines to formulate MWF. Also, the respiratory irritancy of amines and other components found in MWF may be evaluated with the mouse bioassay.

95 URINARY METABOLITES OF RESIN ACIDS IN PERSONS EXPOSED TO AIRBORNE FIBERS FROM A MEDIUM-DENSITY FIBERBOARD FACTORY

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Individuals living in the immediate vicinity of a medium-density fiberboard factory were documented by video and site collections to be regularly exposed to fallout of fiber coming as airborne emissions from cyclones used in the manufacturing process which utilized pine wood chips, urea, and urea-formaldehyde resin. Fibers had been demonstrated by others to be of both respirable and non-respirable size and to contain crystals of the urea-formaldehyde resin. Fiber obtained from a production line inside the factory, as well as, from the property of the exposed individuals was submitted to GC/MS analysis and found to contain several different classes of chemicals: fatty and dicarboxylic acids; monoglycerides of fatty acids; various aromatic aldehydes, acids, esters, alcohols, phenols, and amides; various resin acids and their oxidized derivatives; indoles; and phthalates. Dehydroabietic (I) and abietic (II) acids were principal components. Urine specimens were obtained from the individuals approximately 4 hrs. following a period of documented airborne fiber emissions. GC/MS analysis of these specimens demonstrated the presence of tetrahydroabietic acid (III), 7-ketodehydroabietic acid (IV), and 7-hydroxyabietic acid (V). The presence of IV and V were believed to be due to biotransformation of I and II, respectively, whereas, the presence of III may be due to disproportionation of II during the manufacturing process. These findings coupled with the finding of wood fibers in the sputum of these individuals by others, suggested that the route of entry for these chemicals was *via* respiration of the treated-wood fibers.

96 OLFACTORY EPITHELIUM AND LIVER PEROXISOME PROLIFERATION EVALUATIONS AFTER INHALATION EXPOSURE TO METHYLETHYLKETOXIME

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Male CD-1 mice were exposed 6 hrs/day, 5 days/week for 1, 2, 4 or 13 weeks via whole-body inhalation exposures to methylethylketoxime vapors at 0, 3, 10, 30, or 100 ppm (10/group/interval). Satellite animals were removed after 1, 2, 4 or 13 weeks of exposure and allowed to recover for 4 or 13 weeks (5/group/interval). Nasal turbinates were evaluated microscopically and nasal maps of the lesions prepared. Dose related degeneration of olfactory epithelium lining the dorsal meatus was seen in the anterior region of the nasal cavity, not laterally or posteriorly. Degeneration was present after 1 week but the incidence and severity did not increase with further exposure. The effect was reversible; recovery was complete within 4 weeks at 10 ppm and nearly complete within 13 weeks at 30 ppm. Three ppm was considered to be a NOEL. Using electron microscopy and palmitoyl-CoA oxidation activity evaluations, there was no evidence of any hepatic peroxisome proliferation. Exposure to 30 and 100 ppm however did produce significant increases in levels of hepatic non-protein sulphydryl groups (primarily reduced glutathione).

97 REFORMULATED GASOLINE EXPOSURE ASSESSMENT IN SOUTHEAST WISCONSIN

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The Clean Air Act Amendments (CAAA) of 1990 required that severe ozone nonattainment areas, including the Milwaukee metropolitan area, sell exclu-

sively reformulated gasoline (RfG) beginning on January 1, 1995. In comparison to traditional unleaded gasoline, RfG contains higher concentrations of oxygen to promote more complete combustion and decrease tailpipe emissions of ozone precursors and hazardous air pollutants. Although the CAAA do not specifically mandate the identity of the fuel oxygenates, supplementation of gasoline with the ether based compounds, methyl *tert*-butyl ether (MTBE) and ethyl *tert*-butyl ether (ETBE) has been used extensively to increase the concentration of oxygen in RfG. During the first several months of 1995 over 5000 RfG-related complaints were received. The complaints lead the State of Wisconsin to conduct a health study to investigate health complaints attributed to RfG-exposure and an air monitoring study to determine the concentration of several gasoline constituents in samples collected from different locations in Milwaukee. These samples were collected on several different dates and included ambient sites (24 hr and 2–3 hr collection intervals) and samples collected from the personal breathing zone of individuals while fueling vehicles. Benzene, ethylbenzene, toluene, xylenes (*o*-, *p*-, and *m*-), MTBE, and ETBE were evaluated. While the majority of these constituents were present in the ambient samples collected from most locations, the concentrations were generally low. Exposure to the greatest concentrations of these compounds occurred while refueling, but was considerably lessened when refueling at service stations that had a vapor recovery system. The ambient air concentration of these RfG constituents represents an important component of an exposure assessment, but is not a complete exposure assessment. A complete exposure assessment requires additional information about the individual agents themselved including an estimate of the dose absorbed from inhalation, blood concentrations, and metabolism and elimination of parental compounds.

98 EFFECTS OF 13-WEEK VAPOR INHALATION OF HEXAMETHYLDISILOXANE ON FISCHER 344 RATS

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Hexamethyldisiloxane is a low molecular oligomer used in a variety of commercial applications. To evaluate the vapor inhalation toxicity of hexamethyldisiloxane, a 13-week nose-only inhalation study was conducted in Fischer 344 rats. Five groups of 20 males and 20 females were exposed to target concentrations of 0.13, 0.7, 3.3 and 13.3 mg/l of hexamethyldisiloxane for 6 hours/day, five days a week for 13 weeks. In addition, a high dose satellite recovery group was included and retained for one month following exposure for reversibility, persistence or delayed occurrence of effects. After 13 weeks of exposure, rats were sacrificed and examined for changes in hematology, serum chemistry, urine, selected organ weights, gross and histopathology. No mortality occurred and no apparent treatment-related effects were observed in clinical signs, body weights, food consumption, urinalysis, hematology or clinical chemistry parameters. Increased lung and adrenal gland weights were observed in males in the high dose group and an increase in liver weight was noted in both sexes of this group at the end of the treatment period. Following a one month recovery period, these changes were not apparent. Microscopic findings did not correlate with these organ weight changes. Goblet cell hyperplasia was noted in the nasal cavity of some female rats in this group at the end of the exposure period. Similar findings were not observed in males in this group or in either sex at the end of the recovery period. No other apparent treatment-related microscopic findings were observed in any of the test groups.

99 TWO-WEEK INHALATION RAT STUDY AND *IN VIVO/ IN VITRO* MUTAGENICITY STUDIES WITH PERFLUOROPENTENE-2

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Perfluoropentene-2 is an intermediate in the synthesis of alternative fluorocarbons. A single 6 hr exposure at 5000 ppm caused transient rat convulsions and weight loss. Groups of 10 male rats were exposed for 6 hrs/day for 2 weeks to mean concentrations of 50, 250, or 1400 ppm of vapor followed by a 2-week recovery. Dose-related increased urinary fluoride was suggestive of metabolism. Minimal, reversible, dose-related decreases in leukocytes and lymphocytes were considered significant at 1400 ppm along with a notable decrease in neutrophils. Effects to the respiratory tract in the 1400 ppm group included increased lung weights, slight goblet cell hypertrophy/hyperplasia in the nasal septum, and slight hypertrophy of the bronchiolar epithelial cells. The respiratory effects were reversible after a 2-week recovery. The no-observed-adverse-effect level was considered to be 250 ppm. Ames salmonella assay was negative, but an increase in chromosome aberrations was observed in vitro in human lymphocytes with and w/o activation. Rats exposed at 4000 ppm for 2 days showed bone marrow cytotoxicity but no increase in micronuclei.

100 CLINICAL TOXICOLOGY OF ALIPHATIC HYDROCARBON PROPELLANT ABUSE: UPDATE ON BUTANE

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The intentional abuse of aliphatic hydrocarbon propellants such as butane is an ongoing and serious problem for adolescents of many countries worldwide. Reasons for abuse of butane are many and include: peer group influence, easy availability, low cost, rapid onset of desired effect and rapid cessation of effects. It is the consciousness-altering effect of butane that is desired; the intended, legitimate use of the product is generally not considered by the abuser. Clinical effects of butane abuse include: rapid euphoria, changes in perception, confusion, ataxia, nausea/vomiting, and death. It is highly unlikely that levels of butane required to produce the desired or adverse effects would be encountered during normal use of butane-containing products. Hazardous levels are only achieved by the deliberate concentration of butane vapor by the abuser. Death following deliberate butane abuse has been attributed to asphyxia and cardiac arrest. Inconsistencies in reporting the cause of death can occur because the mechanism of butane toxicity is not well understood. From 1987–1993, there were 43 reported fatalities involving the inhalation of butane; all were attributed to intentional abuse. The mean age of exposure was 16.7 years (range 11–35). An additional 7 fatalities occurred following abuse of aerosol products containing propellant combinations that included butane. In six fatal cases, the individual engaged in some type of physical activity (walking or running) before collapse. During 1992–1993, of the 119 poisoning deaths reported in youths aged 13–19 years, 24 (20.5%) were attributed to the inhalation abuse of hydrocarbons and 14 (11.8%) were attributed to the abuse of butane alone. The scope of morbidity and mortality associated with butane abuse is difficult to assess given the likelihood of under reporting. Few cases of survival following butane-induced cardiac arrest have been reported. Therefore, it is difficult to critically assess the effectiveness of currently available treatment interventions. Identification of clinically-useful treatments will require further study of the mechanism(s) of butane-induced toxicity.

101 RESPIRATORY ABSORPTION OF OCTAMETHYLCYCLOTETRASILOXANE (D4) IN HUMANS DURING ORAL AND NASAL BREATHING

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An understanding of the pharmacokinetics of silicones is essential to characterize potential health effects. To determine the intake and absorption of D_4, 12 healthy subjects ranging in age from 25– 47 years inhaled D4 for one hour which included two 10-minute exercise periods at a workload producing a tripling of minute ventilation. Lung function and blood D4 levels were measured before, immediately after, 1 and 6 hours post exposure. Our target concentration was 10 ppm (120μg/l) and averaged 10.2 ± 0.02 (SEM) ppm (123 μg/l). We continuously measured inspiratory and expiratory D_4 concentrations and the average total intake was 126.5 mg and the apparent uptake was 12.2 mg (9.6%). During rest, the uptake averaged 13% and fell to 7% during exercise. The expired air washout curve indicated an elimination rate of 0.359/min. Blood measurements of D_4 gave a mean peak value of 0.038 μg/ml and indicated a rapid blood clearance ($t^{h-0.62}$). Lung function did not change following D^4 exposure. In 8 additional subjects, we compared D^4 deposition with oral and nasal breathing at resting ventilation. Mean deposition was similar for the two exposure protocols averaging 12.4% after correcting for exposure system losses. These data describing the intake and absorption of D4 should permit more meaningful risk assessment.

102 EVALUATION OF BRONCHOALVEOLAR LAVAGE FROM RATS EXPOSED TO A PYROTECHNICALLY-GENERATED AEROSOL

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Fischer 344 rats (250–300 g) and Hartley guinea pigs (300–400 g) were exposed to the by-products of SFE, a pyrotechnically-generated aerosol fire extinguishant. Exposures levels ranged from 50 g/m^3 (nominal concentration) to 240 g/m^3. The length of exposure was either 15 or 60 minutes. A 700 L whole-body inhalation chamber was used for the exposures and consisted of a supply/exhaust system, aerosol generator and exhaust scrubber. Exposures were conducted under static chamber conditions. The chamber atmosphere underwent aerosol [size (MMAD), distribution (σg) and concentration] and gas [CO, CO_2 and O_2] analysis. Animals were euthanized one hour post-exposure. Half of the animals underwent bronchoalveolar lavage (BAL) and half underwent histopathological examination. The BAL was analyzed for total protein, acid and alkaline phosphotase, β-glucuronidase, lactate dehydrogenase and cell viability. An increase in several of the enzymes were noted as the concentration and length of exposure increased.

103 CALCULATION OF EXPOSURE CHAMBER LEAK RATE WITH THERMAL CORRECTION: A MEASURE OF CHAMBER INTEGRITY

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An existing quantitative standard for exposure chamber integrity based on observed changes in pressure differential was modified to yield calculations of chamber leak rate. The calculations include correction for thermal artifact. Corrected measurement of actual chamber leak rate provides a less arbitrary estimate of chamber integrity than previously used methods. The simple measurements required for the calculations can be readily performed on a daily basis with minimal time consumption and will provide a quantitative measure of potential risks during exposure operations. A series of experiments using controlled leaks demonstrated strong correlation between calculated and measured leak rates, once corrected for thermal artifact. Thermal artifact, in a well-sealed chamber, may be sufficient to cause acceptance or rejection of arbitrarily established chamber integrity operating criteria.

104 SUBCHRONIC NOSE-ONLY INHALATION TOXICITY STUDY IN RATS WITH ATROVENT HFC 134A FORMULATION USING AN AEROSOL GENERATION SYSTEM WITH METERED DOSE INHALERS

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Sprague-Dawley rats were exposed to aerosols of Atrovent HFC 134a Formulation of Ipratropium Bromide (IB) for up to 4 hours/day, 7 days/week for 13 weeks in 52-port, flow-past, nose-only exposure chambers. The aerosols were dispensed with Metered Dose Inhalers using an IITRI-designed aerosol generation system. Twenty rats/sex/group were exposed to 21.9, 41 or 39.2 μg/l IB, or 1.1, 4.0 or 7.5 mg/kg/day of IB, respectively. Additionally, three control groups were exposed to Filtered Air, to the HFC 134a propellant (Vehicle Control), and to a Positive Control consisting of 22.5 μg/l IB or 1.1 mg/kg/day of IB in CFC (chlorofluorocarbon) propellant. Urine from five rats/sex/group was analyzed for ipratropium after 1 day, and 7 and 13 weeks of exposure. Comprehensive gross necropsy and histopathological examinations were preformed on 15 rats/sex/group. No overt signs of toxicity were observed during the study. Statistically significant decreases in body weight and food consumption were observed in the Mid and High dose male rats. Non-concentration-dependent mild hemoconcentration and increased serum albumin levels were observed in IB-exposed animals. At all time points and dose levels, inpratropium was absorbed into the systemic circulation of rats, but did not accumulate. The absorption was proportional to the dose of Atrovent 134a Formulation administered. No gross lesions were observed at necropsy. Histopathologically, a treatment-related increased incidence of chronic suppurative rhinitis in the nasal turbinates was observed in the Mid and High dose animals. Histopathologic changes associated with CFC administration included increased incidence and/or severity and/or increased anatomic distribution of diffuse intracytoplasmic eosinophilic substance within the epithelial cells of the nasal mucosa in one or more anatomic sites and/or levels. Based on these data, the no effect dose level for the Atrovent HFC 134a Formulation was determined to be 1.1 mg/kg/day. These data also indicated that the maximum tolerated dose exceeded the maximum feasible targeted inhaled dose of 9.2 mg/kg/day, achieved under the experimental conditions employed in this study. Additionally, the in-life toxicity, inhalation exposure bioavailability and histopathology for the Atrovent HFC 134a Formulation was comparable or less significant than that of the animals exposed to the Atrovent CFC Formulation.

105 DEVELOPMENT OF A CENTRIFUGAL CONCENTRATOR FOR USE IN AMBIENT PM10 TOXICOLOGY STUDIES

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Epidemiologic studies have provided strong evidence that episodic exposure to ambient particulate matter is associated with increases in morbidity and mortality. These adverse effects have been demonstrated at concentrations far below the NAAQS and, thus, the biological plausibility of these effects has been questioned. For the purpose of exposing test animals to relevant and reproducible exposure concentrations of PM10, we have developed a centrifugal concentrator which can concentrate ambient particles up to 20-fold. A high volume blower is used to deliver ambient air to the inlet manifold and the entrained particles travel along a concentric annulus formed by a stationary solid outer cylinder and a porous inner cylinder rotating at high speed (5K to 15K rpm). Suction applied at one end of the porous shaft causes the dispersion medium (air) to pass through the porous cylinder and into the shaft. Since the rotational velocity of the particles is comparable to that of the rotating cylinder near its surface, the particles move radially outward due to the centrifugal force, as well as down the concentric annulus and inward due to the suction of air into the rotating porous cylinder. The particles reach their highest concentration near the outlet manifold, where they enter the exposure chamber under positive pressure (~0.1" H20). With no particles exiting through the porous cylinder or impacting on the outer cylinder wall (theoretically), the increase in particle concentration is simply the ratio of the flow rates for the inlet air and the exhaust air drawn through the porous cylinder. Using mono- and poly-dispersed fluorescent particles, we have demonstrated that only ~16% of respirable particle mass is lost in the inlet blower and that the Gerber concentrator can deliver as high as 1 mg/m^3 of ambient aerosol to the animal exposure rig.

106 APPLICATION OF AEROSOL STORAGE TO UNIFORM INHALATION EXPOSURE: INITIAL LIMITS OF PRACTICAL OPERATION

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The normalization of inhalation exposure and subsequent inhaled dose among fibers of different chemical composition can be accomplished by sampling fibers stored in the airborne state, thus bypassing the vicissitudes of solid fiber and particle aerosol generation devices. In devices developed in the 1950s, a rotating cylinder of approximately 0.3 aspect ratio (length/diameter) was operated at 2 to 3 rpm to hold particles in the diameter range 1 to 2 μm for periods greater than 48 h with less than 50% loss. Concentration was continuously diluted during extraction of the aerosol by introduction of clean air into the drum. Subsequent theoretical work has shown that 0.4 rpm should be optimal for airborne storage longer than 48 h, assuming that the stored aerosol can be extracted without dilution through movement of one wall. A 750-liter aerosol storage device in the configuration of a horizontal cylinder rotating about its axis was designed to meet the requirements defined from such calculations and still meet inhalation toxicology exposure needs. Initial experiments demonstrated that spheres 2 μm in diameter were retained for periods longer than 17 h with 50% loss when the storage device was operated at 3.5 rpm. Under the same storage conditions, spheres with diameters of 1 μm were retained for periods longer than 48 h with 50% loss.

107 PHYSICAL AND CHEMICAL CHARACTERISTICS OF SFE FIRE SUPPRESSANT ATMOSPHERES IN SMALL vs LARGE SCALE TESTS: IMPLICATIONS FOR PULMONARY DEPOSITION AND TOXICITY

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A comparison of the physical and chemical properties of SFE (a dry powder) fire extinguishant atmospheres was made between those generated to assess

inhalation toxicity in a whole-body exposure system and those generated in facilities used to evaluate fire extinguishment efficacy. The purpose was to establish a basis for extrapolation of laboratory toxicity testing data to field conditions. Nominal concentrations of 50 and 80 g/m^3 in both systems were examined. Mass concentration and concentration decay of the aerosol component, aerosol size distribution and particle growth, and concentration profile of CO and CO_2, the major gaseous components of SFE atmospheres, were determined. At comparable nominal concentrations, the initial mass concentration of SFE aerosols in the large system was 1.5 times greater than that in the exposure chamber. Aerosol concentration in both systems decayed exponentially. Hygroscopic growth of the particles was greater and more rapid in the large system. Gas concentrations in the larger test system also were greater by a factor of 1.5. However, in this system gas concentrations also underwent exponential decay whereas gas concentrations in the exposure chambers were steady state over the 1 hour test periods. Laboratory exposures at the higher target load concentrations produced pulmonary edema and elevated HbCO in rats. Theoretical, mathematical expressions (humans) characterizing CO_2 ventilatory effects on particle deposition and for HbCO formation were derived as a basis for comparison of the potential toxicity of the atmospheres.

108 REFINERY STREAM AND GASOLINE VAPOR GENERATION METHODOLOGY FOR TOXICOLOGICAL EVALUATIONS

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PPSC is performing reproductive/developmental toxicity and neurotoxicity studies on refinery streams that are used in blending fuels, including gasoline. One technical consideration is to generate an appropriate vapor that closely matches the conditions in occupational settings for refinery streams and fuel products. Because extensive data exists on the vapor composition of hydrocarbons from gasoline, we used gasoline distillation parameters to help set conditions to generate test atmospheres of gasoline blending refinery streams. A gasoline sample blended to meet 1990 industry average specifications was provided by the American Petroleum Institute. Sample fractionation was performed using a single-stage open-column distillation process similar in principle to ASTM D86, but using a fractionation splitter to collect the distillate (1:1 reflux). A total of eight fractions was collected. The first fraction was collected from 72° F (ambient) to 100° F and the other seven fractions were collected in 15° F increments from 100–205° F. Analysis of each fraction was performed by gas chromatography. The combined fractions representing the cumulative distillates from 72–130° F had a carbon number distribution of C4-24.8%, C5-51.5%, C6-18.2%, C7-4.3%, and C8-1.2%. The data indicate that the 72–130° F gasoline fraction closely matches the composition of industrial hygiene measurements of consumer and occupational exposures to gasoline vapors. (PPSC consists of Amoco, ARCO, BP America, Chevron, Mobil, Texaco and Unocal.)

109 TIME COURSE AND DIFFERENTIAL EFFECTS OF FUMONISIN ON SPHINGOLIPID BIOSYNTHESIS IN TISSUES AND SERUM OF SWINE

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Fumonisins (FB), mycotoxins produced by *Fusarium moniliforme*, are common contaminants of corn. They affect lung in pigs, kidney in rats and rabbits, and liver in all species. FB inhibits sphinganine (sphingosine) -acyltransferase, resulting in increased sphingoid bases and a decrease in complex sphingolipids. Since this alteration may be the mechanism of FB-induced toxicosis, we examined its time course and organ specificity in pigs fed FB-containing culture material at a pulmonotoxic dose of 20 mg FB_1/kg/day. Pigs were killed on days 0, 1, 2, 3, or 4. Sphinganine (Sa) and sphingosine (So) were measured in lung, liver, and kidney by HPLC as described by Riley, *et al.* (1994), and was modified for serum by combining the extraction and alkaline hydrolysis into one step. Values for Sa and So (pm/mg) are as follows: Day 0: lung = 5, 30; liver = 0.3, 4; kidney = 0.4, 4. Day 1: lung = 23, 44; liver = 20, 10; kidney 49, 16. Day 4: lung = 122, 83; liver = 152, 17; kidney = 162, 26. Serum values followed a similar trend. Thus changes in sphingolipid biosynthesis were evident after 1 day and increased over time. The increase in kidney Sa and So (non-target) was as great as in liver. The lung was least affected, however, it had the highest So values. The role of altered sphingolipid biosynthesis in FB-induced toxicosis needs to be further examined; cell specific changes may be of importance.

110 TOXICITY TO THE LUNG IN PATIENTS WITH PROLONGED EXPOSURE TO ORGANIC SOLVENTS (S)

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Organic solvents (S) such as methyl ethyl ketone, hexane and chlorinated solvents, are commonly used in industrial settings. Inhalation of fumes from (S) may be associated with pulmonary toxicity. Commonly, pulmonary injury is assessed by industrial clinics utilizing spirometry (SP) FVC, FEV1, FEF25–75, and chest x-rays. This study examined 42 patients with a history of exposure to (S) with a mean length of exposure 15.5 years. Patients with a history of alcoholism, drug abuse, heart disease, liver disease and diabetes mellitus and patients on psychotropic medications were excluded. Males and females with a mean age of 46, were included in the study. Subjective symptomatology as reported by the patients (including shortness of breath, cough, chest pain, and wheezing) and comprehensive pulmonary function tests including FVC, FEV1, FEF25–75 and DCLO, metabolic exercise test, methacholine challenge, chest x-ray and CT scan of the chest were done. FVC was reduced in 12.5% of patients tested, FEV1 in 10% FEF 25–75 in 15% and DCLO in 12.8%. Chest x-ray showed abnormalities in 5% and CT of the chest was abnormal in 5.7%. Methacholine challenge testing was pathologic in 42.9% and metabolic exercise testing showed pulmonary pathology in 24%. These objective physiological findings are correlated with the following symptomatology: shortness of breath 88.1%, cough 57.1%, chest pain 31%, and wheezing 4.8%. Our findings here show that 1) exposure to (S) is associated with significant pulmonary symptomatology, 2) this symptomatology is correlated with pathological airway reaction to methacholine and exercise testing, and 3) simple spirometry and chest x-ray were normal in majority of the symptomatic patients. We conclude that (S) can cause occupational lung disease and are toxic to the lung and small airways and must be examined with appropriate technology and 4) the mechanism of toxicity is probably a combination of direct irritant effect of (S) and immunological sensitization.

111 SENSORY IRRITATION STUDIES IN MICE WITH SELECTED CARPET CHEMICALS

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Recently, concerns regarding the quality of indoor air have arisen because of human health complaints and the total amount of time individuals spend indoors each day. Some have attributed complaints such as eye and upper respiratory tract irritation to organic chemicals, especially those emitted from carpets. A mouse model was used to evaluate respiratory irritation potential of a number of carpet emission chemicals. Chemicals were screened at 1000 ppm or at the saturated vapor concentration (if <1000 ppm). When irritation was evident, the concentration causing a 50% decrease in respiratory rate (RD_{50}) was determined. Two alcohols, 1-decanol and 2-(2-butoxyethoxy) ethanol, were not irritating at saturated concentrations. Four chemicals had RD_{50} values between 100 and 1000 ppm (dimethylethanolamine = 100 ppm, propionic acid = 210 ppm, p-chlorotoluene = 500 ppm, o-chlorotoluene = 650 ppm). The other chemicals had less than 50% depression in respiratory rate at 1000 ppm: n, n-dimethylacetamide, 1,1,1-trichloroethane, methylene chloride, isooctane, and mixed xylenes. A risk assessment procedure was applied, taking into account the carpet chemical emission rates (available from other studies), reported human effects from exposure to the individual chemicals, and the RD_{50} data. None of these chemicals were considered likely to cause irritation at room concentrations produced from carpet emissions.

112 SENSORY IRRITATION OF 12 CARPET-ASSOCIATED CHEMICALS AND THEIR POTENTIAL FOR CAUSING IRRITATION AT AMBIENT CONDITIONS

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The sensory irritation potential of 12 chemicals occasionally encountered as emissions in chamber studies of new carpet samples was determined using ASTM E981 with 30 minute exposures. The chemicals/RD50's (ppm) were: 2-chloro-toluene/1019, cyclohexanol/776, propionic acid/684, vinyl acetate/380, 2-butoxyethanol/>900, decane/>1700, 1-decanol/>3, dodecane/>90,

4-ethyltoluene/>1100, isobutanol/>2000, methylene chloride/>1000, 2-methyl-3-hexanone/>1100. (> indicates either vapor saturation or less than 20% respiratory decrease at the indicated concentration). Using a typical room model (10 × 14 × 8 ft, 20 °C, air changes/hour = 0.5), calculated emission rates necessary to achieve a steady state concentration of [0.03 X RD50] for these chemicals were from 2 to 4 orders of magnitude greater than actual emission rates. These chemicals are not expected to cause sensory irritation at ambient conditions using this model. This research was sponsored by the Carpet and Rug Institute.

113 PULMONARY TOXICITY OF COPPER GALLIUM DISELENIDE, COPPER INDIUM DISELENIDE AND CADMIUM TELLURIDE IN SPRAGUE DAWLEY RATS

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Copper gallium diselenide (CGS), copper indium diselenide (CIS) and cadmium telluride (CT) are novel compounds of Group IIIA and IVA elements being used increasingly in the photovoltaic and semiconductor industries. The objectives of this study were to characterize the relative toxicity of these compounds and to evaluate the pulmonary absorption and distribution after intratracheal instillation. A single equimolar dose of CGS, CIS, CT, or saline was administered to female Sprague-Dawley rats by intratracheal instillation. Toxicity endpoints were measured 7, 14, and 28 days after instillation. CGS and CIS had no effect on body, or organ weights. CT caused significant reductions in body weight gain at all time points and spleen weights were significantly decreased at 14 and 28 days after instillation. Relative lung weights were significantly increased in CIS and CT-treated rats at all time points. Microscopic evaluation of liver, kidney and spleen revealed no significant treatment-related lesions. Inflammatory lesions were present in lungs of all treated rats and consisted of an influx of macrophages, lymphocytes, and neutrophils. Inflammation was most severe in CT-treated rats, intermediate in CIS-treated rats and minimal in rats receiving CGS. Hyperplasia of type 2 epithelium was present in lungs of CIS- and CT-treated rats, with the greatest effect in CT-treated rats. Interstitial fibrosis was present in lung of CT-treated rats at all time points. All three compounds caused marked increases in total bronchoalveolar lavage fluid (BALF) cell numbers, with the greatest increase observed in CIS-treated rats. Levels of protein and fibronectin were significantly increased in BALF of all treated animals; levels were highest in CT-treated rats. CIS and CT caused significant increases in lung hydroxyproline, with the greatest increases occurring in CT-treated animals.

114 LEAD-INDUCED CHANGES IN DOPAMINE D_1 SENSITIVITY: MODULATION BY DRUG DISCRIMINATION TRAINING

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To further determine mechanisms of Pb-induced D_1 supersensitivity observed in operant drug discrimination paradigms (Cory-Slechta, Widzowski, 1991), this study evaluated the potential contributions to these effects of: i) synergistic D_1-D_2 receptor interactions, and ii) the effects of the chronic D_1 agonist administration inherent in the drug discrimination procedures themselves. Rats exposed from weaning to 50 or 150 ppm Pb acetate in drinking water and trained to discriminate 6.0 mg/kg of the partial D_1 agonist SKF38393 from saline showed greater sensitivity to SKF38393 (left-shifted dose effect curves) than did the 0 ppm group, confirming the orginal observation of D_1 supersensitivity. D_1/D_2 interactions did not appear to contribute to this Pb-induced D_1 supersensitivity, however, since it was maintained even in the presence of the D_2 antagonist haloperidol. Discontinuation of drug discrimination training procedures and thus of chronic intermittent D1 agonist treatment for 24 days (D_1 agonist washout) resulted in sensitization (left-shift of the dose-effect curves) of control but not Pb-treated rats (i.e., 5 0 ppm), a pattern indicative of Pb-induced D_1 subsensitivity. These data raise questions about the depletion of DA availability as a mechanism of Pb effects on DA systems and suggest that Pb-induced D_1 supersensitivity may represent altered effects of chronic D_1 administration imposed on DA systems modified by Pb exposure per se. ES01247, ES05017, ES05903.

115 PERIPHERAL BENZODIAZEPINE RECEPTORS ARE SENSITIVE INDICATORS OF DOMOIC ACID INDUCED NEUROTOXICITY

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Peripheral benzodiazepine receptors (PBR), normally found in low concentrations in the brain have been shown to increase in response to ischemia and some forms of chemically induced neurotoxicity. It has been proposed that the PBR may serve as a useful indirect biomarker of CNS damage. To further investigate the PBR response in chemical-induced neurotoxicity models, the known neurotoxicant domoic acid was used. Domoic acid has a well documented pattern of pathology and also has been shown to impair spatial learning in the Morris water maze. Adult male Long-Evans rats (225–250g) were given a single i.p. injection of either 1.5mg/kg domoic acid or saline vehicle. Rats were tested daily in the water maze beginning two hours after injection, until the control group reached a pre-set criteria of performance (≤10s to find a hidden platform). Criteria was achieved after 5 days of training. Subsequently, a transferal test (probe) was done and the rats euthanized. A repeated measures analysis of variance revealed that the group treated with domoic acid was significantly impaired in its performance compared to controls ($F_{1,32}=5.7$; $p=0.04$). This difference was confirmed by performance in the probe ($F_{1,8}=16.7$; $p=0.004$). Quantitative autoradiography of [^{3}H]-PK11195 was used to assess neuronal damage. Significant increases in binding were present in the substantia nigra and subiculum as well as the stratum radiatum and stratum oriens of the CA1 subfield of the hippocampus. A non-significant trend toward increased binding was also observed in other limbic structures such as the CA3 region, dentate gyrus and the amygdala, other known targets of domoic acid toxicity. These results further support the utility of the PBR as a sensitive and general marker of chemical-induced neurotoxicity. [Supported by grant Nos. ES07062 to TRG and ES03819].

116 CONCENTRATION-EFFECT RELATIONSHIPS FOR THE EFFECTS OF TRICHLOROETHYLENE ON VIGILANCE IN RATS: EXPOSURE DURATION, CxT PRODUCT, AND PREDICTED BLOOD LEVELS

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The hazard from inhaled volatile organic compounds (VOCs) is presently assessed on the basis of lifetime exposure to the average vapor concentration (C), thus ignoring short-term high-level exposure episodes. This strategy yields rational predictions of risk if the product of C and the duration of exposure (T) yields constant effects on health. The validity of this assumption was evaluated by assessing the acute behavioral effects of inhaled trichloroethylene (TCE) vapor in rats in terms of C, T and estimated arterial TCE concentration ($[TCE]_A$). Adult male Long-Evans rats ($n=11$) were trained to perform a vigilance task in which a press on one retractable lever produced food on trials containing a target (a brief, unpredictable light flash); a press on a second lever produced food on trials lacking a target. Response time (RT) and the signal detection index of sensitivity (SI) were calculated at various times during repeated daily 60-min vigilance tests conducted in air containing 0, 400, 800, 1200, 1600, 2000, or 2400 ppm TCE. Both measures remained stable during test sessions conducted in air. In TCE, SI declined and RT increased as functions of both C and T. Plotting these endpoints as a function of the CxT product showed that SI and RT both depended more on C than on T. $[TCE]_A$ at times of behavioral testing were estimated with a PBPK model for TCE adjusted for fat and liver volumes measured in rats comparable to those being tested. The behavioral effects of TCE were accurately predicted by $[TCE]_A$, and thus appear to depend more closely on the internal dose of TCE (as arterial concentration) than on either its external concentration (C) or cumulative dose (CxT product).

117 PERFORMANCE OF METHYLMERCURY-EXPOSED MONKEYS ON TASKS OF SIMPLE AND COMPLEX REACTION TIME

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Five monkeys (*Macaca fascicularis*) were dosed with methylmercury chloride from birth to 7 years of age. Blood mercury levels during the period of dosing were approximately 0.75 ppm. Previous research in this group of monkeys revealed deficits in visual, auditory, and somatosensory function compared to matched controls. When these monkeys were 20 years old, they

were tested on a series of reaction time tasks of increasing complexity, to assess motor performance and central processing speed. The monkey was required to contact a steel bar, and then release the bar and move the same hand to the appropriate push button to indicate a response choice. The first task measured simple reaction time, followed by complex reaction time tasks of increasing complexity, using multiple pushbuttons and color choices. Central processing speed was assessed by the latency from the onset of the response choice stimulus display to the release of the bar by the monkey, and motor response time was measured by the time between release of the bar and response on the choice pushbutton. Additional measures of performance included variability of response latencies, percent correct choices, and failure to make a choice response. There were no differences between control and treated monkeys on any of these measures as assessed by the intercept term of an orthogonal polynomial regression performed across sessions for each part of the study, although there was an indication of differences in slope for some measures. These results suggest that gross motor speed and intellectual processing speed are not sensitive indicators of methylmercury-induced impairment in aging monkeys, in marked contrast to deficits observed in sensory system function.

118 EFFECTS OF DARK-REARING ON THE SUSCEPTIBILITY OF THE VISUAL SYSTEM TO TRIPHENYL PHOSPHITE-INDUCED NEUROPATHY

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We have shown previously that the visual system of the European ferret first shows signs of triphenyl phosphite (TPP)-induced neuropathy at approximately 5 weeks of age, the time at which eye opening occurs and retinal afferent connections are established. These data suggest that the onset of TPP-susceptibility may be closely related to the developmental state of the visual system and that susceptibility occurs only after some degree of maturation has occurred. In order to test this hypothesis, we administered TPP to visually deprived ferret kits that had been raised for varying periods of time in total darkness. Studies have shown that rearing infant animals in total darkness results in a significant delay in some aspects of normal development of visual system structure and function. We wished to determine if a delay in the functional activation of visual system neurons might also result in a delay in susceptibility to TPP. Ferret kits were raised in total darkness for 6, 8, 10 or 22 weeks after birth. Kits were injected subcutaneously with TPP (1184 mg/kg bw) at 5.5, 7.5, 9.5, or 21.5 weeks of age and killed 4 days later. Brains were processed with the Fink-Heimer silver impregnation technique. Results indicate that the time of onset, extent, and density of degeneration seen in the lateral geniculate nucleus and primary visual cortex of dark-reared kits were similar to those seen in age-matched TPP-injected light-reared controls. Dark-reared kits showed onset of axonal and terminal degeneration in the lateral geniculate nucleus at 5.5 weeks and in the visual cortex at 7.5 weeks with increases in the extent and density of degeneration to adult-like levels by 9.5 weeks. These results indicate that light activation and stimulation is not a necessary prerequisite for the onset of visual system susceptibility to TPP. (Supported by AURIG funds from Michigan State University)

119 EFFECTS OF 3-METHYLINDOLE ON OLFACTORY FUNCTION IN FISCHER RATS

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Numerous chemicals are known to induce olfactory neurotoxicity. However, few studies have objectively evaluated chemical-induced olfactory function deficits in rodents. The purpose of this study was to correlate the nasal histopathologic effects of 3-methylindole (3-MI) with olfactory function as assessed by a behavioral olfactometer. The behavioral olfactometer is a modified shuttle box capable of delivering a diluted odorant, acetaldehyde, to either of two chambers. Adult male F344 rats were trained during 4 daily sessions via a coupled odorant-cued foot shock paradigm. A correct avoidance response was defined as an animal moving to the odorant-free chamber before presentation of the mild foot shock. Rats were dosed after the 4th training session with 0, 100, 200, or 300 mg/kg of 3-MI, ip (n = 4–6 per group). Rats were then tested 6 days later in the behavioral olfactometer. Nasal tissues were collected 7 days after 3-MI administration for histopathologic assessment of olfactory epithelial (OE) damage. Dose-dependent OE damage was observed. Rats in the 300 mg/kg dose group had an almost total loss of OE, whereas small islands of intact OE were present at 100 mg/kg. Decreased body weight and dose-dependent clinical signs of toxicity were observed in rats from all dose groups. However, only the 300 mg/kg dose produced a significant reduction in odor-cued behavior as compared to the control group. These results are in agreement with other studies where extensive damage to the OE is generally required to produce a detectable olfactory deficit. (Supported in part by a grant from the American Petroleum Institute.)

120 ACUTE EXPOSURE TO LOW LEVELS OF TRIETHYL LEAD DOES NOT INHIBIT LTP IN THE ADULT RAT *IN VIVO*

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We examined whether acute exposure to triethyl lead (TEL) modifies the expression of long-term potentiation (LTP) in electrophysiological recordings in the adult rat hippocampus *in vivo*, and compared these results to those seen previously following chronic exposure to inorganic lead. Adult rats were given a subcutaneous injection of TEL at 0, 2.5, or 5.0 mg/kg. The high dose of TEL has previously been reported to cause behavioral changes. Seven days after dosing, animals were anesthetized with urethane (1.2–1.5 g/kg), and prepared for conventional recording of hippocampal field potentials. A bipolar stimulating electrode was lowered into the CA3 region and a glass electrode (0.5–2.0MΩ) was positioned in the CA1 region until a maximum response to the CA3 stimulus was observed. LTP was induced by tetanic stimulation (one 400ms train of five 50ms pulses at 50Hz), and recordings of field potentials were repeated at 0.5, 1, 2, and 4 hours post-tetanus. TEL appeared to have no effect on LTP when compared to that of the controls. The increase in the population spike (PS) amplitude was observed to persist for 4 hours, and was similar to the increase in PS observed in control animals. Similarly, the EPSP height and slope appeared unaffected by TEL. In a previous study, we observed that both 500 and 1000 ppm of lead acetate completely inhibited LTP at 4 hrs post-tetanus. These data suggest that TEL and inorganic lead neurotoxicity may be mediated through different mechanisms.

121 DEXAMETHASONE TREATMENT DURING GESTATION HAS LONG LASTING EFFECTS ON STRESS AND MEMORY

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One of the primary functions of endogenous glucocorticoids is the induction of metabolic preparatory measures to assist the body's innate stress response to "flight or fight". However, long term exposure to glucocorticoids has proven to cause detrimental effects including neuronal death. Dexamethasone (Dex), a potent synthetic glucocorticoid is used for long periods of time to treat medial conditions despite this, including during pregnancy. Therefore, we were interested in the effects of the drug during development; in particular the long term effects on the stress hypothalamic pituitary adrenal axis (HPA) during fetal and postnatal life. We focused on: 1) Long lasting effect of prenatal Dex treatment on hippocampal function & 2) Long lasting effects of prenatal Dex treatment on HPA axis activation and inhibition to an acute stressor. To address these aims, pregnant rats were given oral Dex (2.5μg/ml:0.03% ethanol:saline) water on day 15 of gestation until delivery. Control animals received equivalent concentration of ethanol:saline water (Sal). Litters were randomized, cross-fostered and reduced to 12 pups. Four groups describing the maternal: offspring prenatal treatment resulted: Dex-Dex; Dex-Sal; Sal-Dex; and Sal-Sal. Once adults, animals were submitted to water maze testing to assess hippocampal function and restraint to test acute response to stress. We found that animals exposed to Dex during gestation, had poor spatial memory acquisition. Dex exposed animals had better termination of stress when compared to all other groups. We conclude that occupation of hippocampal glucocorticoid receptors during gestation has long lasting effects on memory functions. However exposure to glucocorticoids during this same periods of time appears to be beneficial in the development of a competent HPA axis.

122 METHYL t-BUTYL ETHER- (MTBE-) INDUCED CENTRAL NERVOUS SYSTEM (CNS) DEPRESSION: EFFECTS OF METABOLISM OF THE COMPOUND

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The gasoline additive MTBE causes CNS depression. It undergoes P450-dependent O-demethylation to t-butyl alcohol (TBA) and formaldehyde (FA) as primary metabolites. To assess the effect of biotransformation on the CNS depressant effects of MTBE, we measured the duration of (i) ataxia (assessed by roto-rod performance) and (ii) hypnosis, following a single sub-hypnotic (0.5 mg/kg) and hypnotic (1.2 mg/kg) i.p. dose of MTBE, respectively, in male Fisher 344 rats pretreated with either of the following P450 inducers: phenobarbital (PB), beta-naphthoflavone (BNF) clofibrate (CF), or vehicles (controls). Pretreatment with PB, and to a lesser extent CPIB but not BNF, prolonged the duration of ataxia or narcosis from MTBE. Similarly, only PB and CF induced hepatic microsomal CYP2B1/2 (the major MTBE O-demethylase), as well as MTBE O-demethylation. Pretreatment with P450 inducers alone effected neither ataxia nor narcosis in the animals. The plasma concentration of TBA following the hypnotic dose of MTBE was elevated four-fold by PB-pretreatment. The data suggest that metabolic status is a major potential determinant of sensitivity to the central effects of MTBE

123 BRAINSTEM AUDITORY EVOKED RESPONSE AND PRENATAL LEAD EXPOSURE: PERSISTENT EFFECT OF MID-PREGNANCY MATERNAL LEAD AT 5 YEARS

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In a previously published report (Neurotoxicol 15(3): 695–700; 1994) with a subset of the Mexico City Prospective Lead Study cohort we demonstrated a highly significant positive correlation between maternal blood lead level at 20 weeks of pregnancy and the Peaks III-V conduction time of the brainstem auditory evoked response (BAER) in the first month of life. We present here the preliminary results of BAER testing in the older children of the cohort, from 5–7 years of age. Maternal blood lead at 20 weeks of pregnancy was significantly associated with Peaks III-V conduction time of the BAER of the older group. The BAER association with lead was significant in both simple and multiple regression analyses. In contrast to the younger group, the correlation was negative. No other prenatal or postnatal blood lead level was significantly associated with either BAER latency or conduction time at the click presentation rate of 11/sec. The persistent alteration of the III-V conduction time with mid-pregnancy maternal lead exposure suggests that prenatal lead exposure at a time of rapid growth of the auditory brainstem CNS may produce lasting changes in auditory function associated with those structures.

124 PRECLINICAL SAFETY STUDIES FOR CYCLIC [D-PENICILLAMINE[2] - D-PENICILLAMINE[5]] ENKEPHALIN (DPDPE), A DELTA OPIOID ANALGESIC DELIVERED INTRATHECALLY

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The δ-opioid peptide cyclic DPDPE is a potent analgesic after spinal delivery in animals. Prior to initiation of large animal studies and subsequent clinical evaluation, safety studies examining the effects of repeated intrathecal delivery in a well defined rat model were accomplished. Male Sprague Dawley rats (275–300 gms) were prepared under halothane anesthesia with catheters inserted intrathecally from the cisterna magna to the lumbar enlargement (day -5). Starting day 1, rats were randomly assigned to receive daily intrathecal injections for 4 days of the highest physiologically tolerable dose (100 μg/10μl; N = 10) or an equal volume of saline (N = 5). On day 6, rats were anesthetized and underwent perfusion clearance and fixation. Spinal cords and meninges were examined at three levels in a blinded fashion. All rats showed normal motor function and behavior throughout the study period. DPDPE- but not saline-treated rats showed expected antinociception as measured by the 52.5°C hot plate test. Histopathological evaluation revealed no significant differences in cell morphology, meningeal inflammation or myelination between the DPDPE or saline-treated rats. These studies indicate that a maximum tolerable dose of DPDPE produces no spinal toxicity in rats after repeated intrathecal administration. (Funded by Rita Allen Foundation)

125 INCREASED VULNERABILITY TO THE TOXIC EFFECTS OF COLCHICINE DURING OPTIC NERVE REGENERATION IN GOLDFISH

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Goldfish visual function recovers after optic nerve crush (ONC). The regeneration of retinotectal pathways can be studied behaviorally by utilizing a branchial suppression response conditioned to a light stimulus. The capacity of the retinal ganglion cells (RGC's) to regenerate the optic nerve can also be studied *in vitro*; the ONC induces neurite outgrowth from the RGC's, which can be quantitated in retinal explant cultures. The role of microtubule polymerization during regeneration was investigated by intraocular administration of the microtubule depolymerizing drug colchicine (COL). Fish injected from 0 to 3 days post ONC had delayed visual recovery, while fish injected with COL after 3 days but before 21 days post ONC did not recover visual function. The retinal explant experiments showed that intraocular COL inhibited the normally observed ONC-induced neurite outgrowth. Furthermore, goldfish injected with COL at the time of ONC were able to regain the ability to grow neurites in culture while fish injected at five days post ONC did not. These experiments taken together indicate an increased period of vulnerability of the regenerating visual system that begins 3–5 days post ONC. These experiments also indicate that the site of COL's toxic effects is in the retina, presumably in the RGC's. This previously undescribed vulnerable period is currently under investigation.

126 LEAD-INDUCED CHANGES IN NMDA RECEPTOR COMPLEX BINDING: CORRELATIONS WITH LEARNING ACCURACY AND WITH SENSITIVITY TO LEARNING IMPAIRMENTS CAUSED BY MK-801 AND NMDA

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To further evaluate mechanistic relationships between glutamatergic system biochemical and behavioral toxicity, Pb-induced changes in MK-801 and CGP binding sites in 4 brain regions (frontal cortex, dentate gyrus, CA1 and striatum) were correlated with changes in learning accuracy and with sensitivity to the accuracy-impairing effects of MK-801 and NMDA on a multiple repeated acquisition and performance schedule. Data came from a single population of rats exposed from weaning to 0, 50 or 250 ppm Pb acetate in drinking water. Pb decreased levels of MK-801 binding and increased levels of CGP binding, effects statistically significant in some brain regions but exhibiting similar trends across regions. At 0 ppm, increased numbers of MK-801 and CGP sites were correlated with increased learning accuracy levels but with decreased accuracy levels in response to MK-801 or NMDA. These correlations were negated by 50 ppm Pb exposure and actually reversed by 250 ppm Pb, an effect that could be attributable to an alteration of NMDA receptor complex subunit configuration and thus the efficacy of ligand binding. These data provide additional support for an involvement of NMDA receptor complex in Pb-induced learning imapriments. These effects were noted most frequently in striatum raising the possibility that dopamine-glutamatergic interactions contribute to Pb's effects. ES01247, ES05017, ES05903.

127 ACTION OF METHYLMERCURY (MeHg) ON $GABA_A$ RECEPTORS IS RESPONSIBLE FOR ITS EARLY APPARENT STIMULATION OF HIPPOCAMPAL CA1 SYNAPTIC TRANSMISSION

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Previous results showed that acute bath application of MeHg caused a concentration-dependent biphasic effect on synaptic transmission in the CA1 region of hippocampal slices. Specifically, an initial period of stimulation of synaptic transmission preceded the later block of transmission induced by MeHg. Moreover, MeHg blocks inhibitory postsynaptic potentials (IPSPs) more rapidly than it blocks excitatory postsynaptic potentials (EPSPs). To identify if the early increased phase is due to action of MeHg at $GABA_A$ receptors, extracellular recordings of population spikes and intracellular recordings of EPSPs were compared with or without pretreatment of hippocampal slices with bicuculline, a $GABA_A$ antagonist. In the absence of bicuculline, MeHg (100 μM) first increased and then decreased amplitudes of both population spikes and EPSPs to complete block. After pre-incubation of slices with 10

μM bicuculline, MeHg only decreased the amplitudes of population spikes and EPSPs to block; no early increased phase of synaptic transmission occurred. To test whether MeHg-induced alterations of the resting membrane potentials of CA1 neurons play a role in these biphasic effects, the effect of MeHg on excitatory postsynaptic currents (EPSC) was examined using the single-microelectrode voltage-clamp technique. When CA1 neurons were voltage-clamped at their resting membrane potential levels, MeHg still caused an increase prior to the decrease in EPSC amplitudes. However, after pretreatment of slices with bicuculline, MeHg no longer caused an increase in EPSC amplitude, but still decreased amplitudes of EPSCs to block. Thus, block by MeHg of $GABA_A$ receptors results in disinhibition of excitatory hippocampal synaptic transmission, and is evidently responsible for the initial excitatory effect of MeHg on hippocampal synaptic transmission. Supported by NIH grant ES03299.

128 LEAD (Pb) IMPAIRS SPATIAL LEARNING IN THE MORRIS WATER MAZE: STUDIES USING CHRONIC DEVELOPMENTAL EXPOSURE AND ACUTE INTRA-HIPPOCAMPAL MICROINJECTION

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The neurobehavioral toxicity of lead (Pb) was investigated by conducting tests of spatial learning in the Morris water maze. Continuous exposure of rats to 0 or 250 ppm Pb acetate *in utero*, during lactation, and in the diet after weaning resulted in significant deficits in water maze performance in both reference and working memory paradigms when testing began at postnatal (PN) day 21. No significant differences were observed when testing began at PN56 or PN91. To test whether Pb-induced behavioral deficits could be observed in normal adult rats, we examined spatial learning after direct acute administration of 100 μM Pb acetate or sodium acetate by bilateral microinjection into the dorsal hippocampus (1 μl each side). Significant deficits in reference spatial learning were detected in the Pb-treated group when Pb was administered at the onset of behavioral testing. However, no significant differences between treatment groups were detected when Pb was administered after rats were allowed to acquire the spatial learning task, nor when the platform position was reversed to the opposite side of the water maze. These results indicate that developmental Pb exposure caused deficits in the Morris swim task. The observation that these effects were only detected in younger rats (PN21) is consistent with previous research demonstrating that children are more sensitive to Pb-induced impairment of cognitive function than adults. Additionally, we have shown that intrahippocampal administration of Pb caused deficits in the acquisition, but not recall of spatial location in the Morris swim task. This suggests that Pb may interfere with hippocampal function directly, and exposure during the development of the CNS may not be a prerequisite for the expression of behavioral effects. This research was supported by ES06189; ES03819, and F32NS09573 to DAJ.

129 FURTHER USE OF A BEHAVIORAL TEST BATTERY TO ASSESS RHESUS MONKEY OFFSPRING EXPOSED TO COCAINE IN-UTERO

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In order to explore possible long-term effects of gestational cocaine (COC) exposure, pregnant rhesus monkeys were treated with COC chronically from about 1 month (mo) of gestation until term (either 0 (N = 3), 0.3 (N = 3), 1.0 (N = 3), or escalating doses up to 8.5 (N = 3) mg/kg (i.m.), 3 times/day, 5 days/week). Begining at 6 mo of age, the behavior of offspring (N = 12) was monitored using an operant test battery with 4 food-reinforced tasks designed to model aspects of motivation, learning, color and position discrimination, short-term memory and attention. Between 6 and 18 mo of age, the acquisition of operant behaviors by juvenile offspring was not differentially affected by gestational COC exposure (at 18 mo: F = 0.83, 0.50, 0.23, 1.02; all $p>0.05$). Possible long-term effects of gestational COC exposure were examined further by measuring the sensitivity of acquired operant behaviors in adolescent offspring to acute administration of amphetamine (AMP: 0, 0.03, 0.1, 0.3, 1.0 or 3.0 mg/kg (i.v.)) or haloperidol (0, 0.001, 0.003, 0.01 or 0.03 mg/kg (i.v.)). When assessed at 37 or 41 mo of age, the operant behavioral responses of offspring to acute AMP or HAL challenges were not differentially affected by gestational COC exposure (AMP at 37 mo: F = 1.70, 0.92, 2.87, 2.18; HAL at 41 mo: F = 3.85, 0.56, 0.29, 0.63; all $p>0.05$). It was concluded that gestational COC exposure had no significant effect on the subsequent acquisition of complex behaviors by offspring, or the sensitivity of those behaviors to pharmacological challenge with AMP or HAL. Supported by NIDA IAG #224-89-003.

130 DEVELOPMENTAL EXPOSURE TO AROCLOR 1254 PRODUCES HEARING DEFICITS AS ASSESSED BY BRAINSTEM AUDITORY EVOKED RESPONSES (BAERS) IN ADULT RATS

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Developmental exposure to Aroclor 1254 (A1254) has been previously reported to produce an increase in the threshold for detecting a low frequency tone using reflex modification audiometry (Goldey et al., Toxicol. Appl. Pharmacol., In Press). We recorded brainstem auditory evoked responses (BAERs) from a subset of the male animals to help localize the site of auditory dysfunction. Dams were administered A1254 (0,1,4, or 8 mg/kg/day; po) from GD6 through PND 21. At approximately 364 days of age, male offspring had BAERs recorded using 1 (65 and 80 dB), 4 (60 and 80 dB), 16 (40 and 80 dB), and 32 kHz (40 and 80 dB) filtered clicks. When the 1 kHz stimulus was used, A1254 decreased the amplitude of peaks $P_{1A}N_1$ and $P_{1B}N_1$, N_1P_2, and increase the latency of peak P_4. The decreased amplitudes of peaks $P_{1A}N_1$ and $P_{1B}N_1$ were observed at doses of A1254 as low as 1 mg/kg/day. When the 4 kHz stimulus was used, the amplitudes of peaks N_1P_2 and P_2N_2 were decreased by exposure to A1254. No significant changes in BAERs were observed for the 16 or 32 kHz stimuli. The data indicate that developmental exposure to A1254 resulted in a permanent low- to mid-frequency auditory deficit, which may be localized to peripheral auditory structures (cochlea and/or auditory nerve).

131 INHIBITION OF EXCITATORY POST-SYNAPTIC POTENTIALS IN RAT HIPPOCAMPAL SLICES BY 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (2,3,7,8-TCDD) AND 2,2',5,5'-TETRACHLOROBIPHENYL (2,2',5,5'-TCB)

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Human epidemiological and laboratory animal studies show that halogenated aromatic hydrocarbons (HAHs), such as polychlorinated biphenyls (PCBs) and polychlorinated dibenzodioxins, alter cognitive function and learning. The cellular basis of HAH-induced alteration of brain function is not well-understood. The brain region known as the hippocampus is a likely site of toxic action because of its well-known roles in learning and memory, as well as its propensity to accumulate environmental neurotoxicants. A hippocampal function that can be measured readily is evoked excitatory post-synaptic potentials (EPSPs), which are an index of excitatory synaptic function. In this study, effects of HAHs on EPSPs were characterized in hippocampal slices from male Sprague-Dawley rats. 2,3,7,8-TCDD and 1,2,3,4-TCDD were used because these HAHs are prototypical potent and weak aryl hydrocarbon receptor (AhR) agonists, respectively. 2,2',5,5'-TCB was used as a prototypical ortho-substituted PCB which acts through AhR-independent pathways. For each slice, peak amplitudes of EPSPs during a 15 minute recording period (1 recording/minute) were averaged and used as baseline (100%). Subsequent EPSPs were expressed as percentage of baseline. 2,3,7,8-TCDD and 1,2,3,4-TCDD did not alter EPSPs in slices from the middle third of the hippocampus. However, in ventral slices, 2,3,7,8-TCDD significantly decreased EPSPs (55.10 ± 9.07% at 5 minutes), whereas 1,2,3,4-TCDD was inactive. 2,2',5,5'-TCB decreased EPSPs in both middle (57.28 ± 13.13%) and ventral slices (63.76 ± 6.98%). We have previously shown that 2,3,7,8-TCDD stimulates transient Ca^{2+} uptake in primary cultures of hippocampal neurons, which was blocked by L-type calcium channel blocker, nifedipine. In this study, nifedipine blocked 2,3,7,8-TCDD-induced inhibition of EPSPs. These results suggest that HAH-induced inhibition of EPSPs may be mediated by L-type calcium channel activity. TCDD- and PCB-induced inhibition of EPSPs are now being used as a model for investigating HAH-induced neurotoxicity.

132 QUANTITATIVE ELECTROENCEPHALOGRAPHY (QEEG) METHOD VALIDATION WITH TRIMETHYLTIN IN FISCHER 344 RATS

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This study was conducted to validate qEEG test methods in unanesthetized rats, using trimethyltin chloride (TMT) to produce brain lesions. Neurobehav-

ioral tests and micropathology of neural tissues were used for comparison with qEEG findings to establish the relative sensitivity and utility of the qEEG test methods. Nominal doses of 0, 4, 8 and 12 mg/kg TMT were administered i.p. to young-adult male and female Fischer 344 rats. Animals were evaluated daily for clinical signs and subjected to FOB and motor activity (figure-8 maze) testing on day 5. qEEG was performed on day 6 and rats were anesthetized and sacrificed by perfusion with Universal fixative on day 7 post-treatment.

Treatment-related clinical signs were evident in high-dose males and mid- and high-dose females. FOB findings were evident in high-dose males and females but there was no effect on activity in either sex at any dose. qEEG changes and micropathology associated with treatment were detected in both sexes, with significant findings at all doses. The following qEEG changes associated with TMT occurred in males and females at all dose levels: increased total power (TP), relative delta (RD), absolute delta (AD), theta (AT), alpha (AA) and amplitude in the theta-beta range (AT-B) frequencies. There was also a decrease (left shift) in median spectral edge frequency within the theta-beta range (MT50) and spectral edge frequencies at the 50th and 90th percentile (SF50 and SF90). The auditory, visual and somatosensory stimulus conditions appeared to provoke electrophysiological effects at the low dose in both sexes that were not detected under resting, normotonic conditions. Minimal neuronal necrosis in the hippocampus was evident at the low dose in both sexes. These results establish that these test methods are useful for the detection of brain lesions produced with a neuropathic agent.

133 METHYLMERCURY (MeHg) DECREASES WHOLE CELL BARIUM CURRENT (I_{Ba2+}) IN CEREBELLAR GRANULE NEURONS

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MeHg disrupts neuronal Ca^{2+} homeostasis through numerous pathways, including block of voltage-gated Ca^{2+} channels. In synaptosomes and PC12 cells MeHg decreases $^{45}Ca^{2+}$ uptake, and rapidly and irreversibly decreases Ba^{2+} current, respectively. We examined the ability of MeHg to interact with Ca^{2+} channels in a sensitive *in vivo* target of MeHg, cerebellar granule cells in primary culture. Using whole cell voltage clamp with 20mM Ba^{2+} as charge carrier, 250 ms depolarizations to potentials ranging from -20 to +40mV elicited currents which reached a peak at 0 or 10 mV and decayed to a non-zero value. MeHg blocked I_{Ba2+} at all potentials tested, with no alteration in leakage current. The effect at 1μM was slightly larger at the more negative potentials, while 5 and 10 μM MeHg blocked current equally well at all potentials tested. 250 ms depolarizations to 0mV elicited currents which exhibited both inactivating and non-inactivating components. Measurement of I_{Ba2+} elicited within the first 35ms after the voltage step (peak) and that elicited at the end of the voltage step (end) showed that MeHg blocked both components to a similar extent in a concentration- and time-dependent manner. Peak current was blocked 46% and 59% following a 2 min exposure to 1 or 5 μM, respectively, while 10μM blocked 58% of peak current after 40 sec. End current was blocked approximately 60% following similar exposures at each concentration. Increasing the frequency of stimulation from 0.1 to 0.2 and 0.4 Hz facilitated block of Ba^{2+} current by MeHg, particularly at the higher concentrations. Supported by NIH grants R01-ES03299 and T32-ES07255.

134 ALTERATIONS IN THE REGULATION OF RETINAL DOPAMINE CONTENT AND RELEASE FOLLOWING LEAD EXPOSURE DURING DEVELOPMENT

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Following developmental and/or postnatal lead exposure in rats and monkeys, electroretinographic (ERG) alterations in dark and light adaptation occur (Otto and Fox, 1993) and regional brain levels of dopamine (DA) and its metabolites dihydroxyphenylacetic (DOPAC) and homovanillic acid (HVA) are decreased (Lasley et al., 1992; Kala and Jadhav, 1995). Similar changes in retinal DA metabolism could account for the ERG alterations. Therefore we measured retinal [DA], [DOPAC] and [HVA] in dark-and light-adapted adult rats following low or moderate level lead exposure during development (days 0–21). [DA] in controls was similar during dark and light adaptation whereas [DOPAC] and [HVA] increased 93–196% during light adaptation. These latter effects are due to increased tyrosine hydoxylase activity and DA turnover. In lead-exposed rats, relative to controls, [DA] significantly decreased during dark and light adaptation (≤49%) whereas [DOPAC] and [HVA] were decreased significantly only during light adaptation (≤60%). These dose-dependent, long-term alterations in the regulation of retinal DA content and turnover are similar to those found in selective brain regions of lead-exposed rats. Thus, our data further support the idea that lead impairs DA metabolism and extend these findings by delineating a possible mechanism underlying the ERG dark and light adaptation deficits. Our preliminary biochemical findings, consistent with the above results, reveal DA supersensitivity in rod photoreceptors isolated from similarly lead-exposed rats. Supported by NIH Grant ES03183 and ATSDR/MHPF Grant U50/ATU 398948.

135 TOLUENE REDUCES OUTER HAIR CELLS LENGTH AND ELEVATES $[Ca^{2+}]i$ IN COCHLEAR CELLS

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Toluene causes mid-frequency hearing loss in laboratory studies suggesting that toluene preferentially impairs apical-middle turns where midfrequencies are encoded. OHCs are contractile auditory sensory cell that modulates the sensitivity of the hearing. We have studied whether toluene preferentially impairs motility of outer hair cells (OHCs) from apical-middle turns of cochlea rather than from the base. The effects of toluene on the $[Ca^{2+}]i$ of OHCs and primary auditory afferent neurons, the spiral ganglion cells (SGCs) were also be studied. Cells were collected from guinea pigs. For morphologic study, the organ of Corti from different turns were dissected and triturated, then examined under a microscope fitted with a CCD video camera. The length was measured for 60 min with 0, 30μM, 100μM, and 7mM toluene. For $[Ca^{2+}]i$ study the organ of Corti was incubated in FURA-2 AM, and then studied using a digital imaging fluorescence microscope. Dynamic changes in $[Ca^{2+}]i$ were monitored during toluene exposure both when Ca^{2+} was present extracellularly and when it was absent. Toluene promotes significant reductions in OHC length that are greater in OHCs from the apical-middle turn. Toluene also elevated $[Ca^{2+}]i$ levels in SGCs and OHCs both in Ca^{2+} and Ca^{2+} free condition. The results demonstrate that hearing loss induced by toluene may result from interfering with of OHC motility and homeostasis of $[Ca^{2+}]i$ in both the outer hair cell and spiral ganglion cell. Supported by NIH grant ESO2852.

136 EVALUATION OF INTERFERON-INDUCED DEPRESSION USING THE FORCED SWIMMING TEST IN MICE

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Human interferon (huIFN) has been reported to induce several CNS side effects such as drowsiness, fever, sensory hypersensitivity, motility disorder and depression. We investigated huIFN-induced depression using the forced swimming test in mice. HuIFN-alpha (6×10^2 – 6×10^4 IU/kg, i.v.) dose-dependently increased the immobility time in the forced swimming test, but huIFN-beta and -gamma, as well as the mouse-IFNs (mIFN-alpha/beta, -beta and -gamma, 3×10^6 U/kg, i.v.), did not affect it. The increase in the immobility time induced by huIFN-alpha was reversed by pre-treatment with imipramine (30 mg/kg, i.p.), anti-depressant. Naloxone (opioid receptor antagonist), naloxonazine (mu_1-antagonist) and beta-funaltrexamine (mu_1 & mu_2-antagonist) inhibited the effect of huIFN-alpha, but naltrindole (delta-antagonist) and nor-binaltorphimine (kappa-antagonist) did not. These results suggest that huIFN-alpha has a greater potential for inducing depression than huIFN-beta and -gamma, that anti-depressants are effective against huIFN-alpha-induced depression, and that the effects of huIFN-alpha are mediated by mu-opioid receptors but not by IFN receptors.

137 LEAD-INDUCED CHANGES IN DOPAMINE D_2 AUTORECEPTOR SENSITIVITY: MODULATION BY DRUG DISCRIMINATION TRAINING

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This study sought to further determine mechanisms of Pb-induced D_2 autoreceptor supersensitivity observed in operant drug discrimination paradigms (Cory-Slechta and Widzowski, 1991), specifically, the potential contribution of the effects of the chronic intermittent D_2 agonist administrations inherent in the drug discrimination procedures themselves. Rats exposed from weaning to 0 or 50 ppm Pb acetate in drinking water were trained to discriminate 0.05 mg/kg of the D_2-like agonist quinpirole from saline. Dose-effect curves

for quinpirole sensitivity were redetermined at three different time points across a period of 10 months. Sensitivity to quinpirole did not differ in control and Pb-treated rats initially, as no differences in the quinpirole dose-effect curves were found in either curve 1 or 2. However, curve 3 revealed a significant enhancement of quinpirole sensitivity by Pb. This was due to the systematic left-shift of the 50 ppm quinpirole curve across successive redeterminations while the control curve showed no consistent shifts. These findings suggest that Pb-induced D_2 autoreceptor supersensitivity arises from differential effects of chronic intermittent D_2 agonist administration imposed on biochemical alterations of DA systems produced by Pb alone. ES01247, ES05017, ES05903.

138 CONCENTRATION - DURATION RELATIONSHIPS FOR INHALED TRICHLOROETHYLENE (TCE) ON RAT VISUAL FUNCTION

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Current risk assessment practices assume lifetime exposures, and do not address the risks of short term excursions above the exposure standards. These experiments explored the relationship between exposure concentration (C) and duration (T) for acute exposure to TCE. The null hypothesis was that the product of CxT would produce a constant health effect (Haber's Law). Awake, adult, male Long-Evans (L-E) rats were exposed to TCE vapors in a head-only exposure chamber while pattern onset/offset visual evoked potentials (VEPs) were recorded. CxT products of 0 ppm-hr (0 ppm-4 hr) or 4,000 ppm-hr were created including: 1,000 ppm-4 hr; 2,000 ppm-2 hr; 3,000 ppm-1.3 hr; and 4,000 ppm-1 hr (n=9–10/condition). A physiologically-based pharmacokinetic (PBPK) model was developed for L-E rats and used to simulate arterial blood concentrations (Ca). Peak Ca predicted by the model correlated well with C but not with CxT. Likewise, amplitude of the VEP frequency double component (F2) decreased as a function of C and Ca, but not T or CxT product. Thus, Haber's Law did not hold, but estimated peak Ca was a good predictor of acute neurotoxic effect.

139 SENSITIZATION INDUCED BY SUB-CONVULSIVE DOSES OF THE NOVEL NEUROTOXICANT TRIMETHYLOLPROPANE PHOSPHATE (TMPP)

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Last year we presented data demonstrating that doses of TMPP lower than 0.25 mg/kg (ip), that yield no convulsive behaviors or other casually observed behavioral indication of altered neural activity, modulated responding on a number of behavioral tasks and induced long-term behavioral sensitization to an amphetamine challenge. This year we extended these findings by demonstrating that sub-convulsive doses of TMPP are capable of inducing long-term and time-dependent sensitization in (1) acquisition of schedule-induced polydipsia (SIP) as well as in (2) appetitive reinforcer approach (ARA) duration. Male Long-Evans and/or male Wistar rats weighing 300–500 g were used. Thirty days before SIP testing, rats were injected (ip) with either 0.20 mg/kg of TMPP or 0.20 ml/kg vehicle three times/week for three weeks. Thirty days following the final injection the animals were evaluated for SIP acquisition. 22.5-hr food deprived rats were tested daily in a procedure in which 45 mg food pellets were unconditionally delivered at a frequency of 1 pellet/min. for a 1-hour session in a standard operant chamber. TMPP reliably reduced the number of SIP sessions necessary to induce asymptotic drinking levels. For ARA testing, rats were injected (ip) with TMPP (0.10 or 0.20 mg/kg) or vehicle equivalent on several different schedules. Fourteen to thirty days following the last injection rats were placed in a modified open-field chamber that contained 'fenced off' raw hamburger meat in two adjacent corners of the chamber. TMPP reliably increased the sniffing duration for the hamburger reinforcer. These results suggest that TMPP produces physiological changes that persist much longer than the pharmacological effect of the compound.

140 SELECTIVE CNS TOXICITY OF DIHYDROFURAN ANALOGUES

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One-week pilot inhalation studies were conducted with 2,5-dihydrofuran (DHF) and 2,3-DHF to determine exposure concentrations for 28-day studies. Male and female CD rats were exposed to concentrations of 1250, 2000, or 3700 ppm of each substance for 6 hr/day for 5 days. Animals were observed before, during, and after exposure for signs of toxicity. Body weights were measured on Days 0, 2, and 4. Several animals exposed to 2,5-DHF exhibited convulsions and abnormal chewing activity during or 18 hours after exposure to 2000 or 3700 ppm. Exposure to 1250 ppm for 5 days resulted in CNS depression during exposure, but no other signs of toxicity. Animals exposed to 2,3-DHF showed no evidence of toxicity other than CNS depression during exposure. In a subsequent 4-week study of 2,5-DHF, chewing motions and convulsions were observed in animals exposed to 1250 ppm. Gait was abnormal, but there were no changes in grip strength. Response to touch was slightly lessened after 4 weeks. No neurotoxic signs were observed in animals exposed to 125 or 400 ppm 2,5-DHF. There were no neurohistopathologic changes in the CNS and PNS of animals examined following 4 weeks of exposure. Although 2,5-DHF has some structural similarity to a cyclized derivative of 2,5-hexanedione, there were no signs of peripheral neuropathy observed in an abbreviated FOB.

141 NEUROBEHAVIORAL EFFECTS OF CHRONIC EXPOSURE TO SCOPOLAMINE IN FISCHER 344 RATS

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This study was designed to characterize the neurobehaviorial effects of long term exposures to scopolamine, an antimuscarinic agent known to elicit CNS effects. Groups of 10 F344/N rats/sex received daily doses of 0, 1, 5, or 25 mg/kg scopolamine by oral gavage for 2 years. Body weights and measures for grip strength, pawlick latency, motor activity, and startle response were recorded on days 1, 90, 180, 270, 360, and 720. Passive avoidance training occurred on day 90 and retention scores (latencies) were measured on days 91, 180, 270, 360, and 720. Body weights were depressed relative to controls in the 25 mg/kg group. Three out of 5 behavioral parameters were marginally affected by scopolamine (occurred sporadically and limited to the 5 and 25 mg/kg groups). Although treatment related hyperactivity, an attenuated startle response, and lower passive avoidance latency scores conform to the acute effects of scopolamine on rodent behavior, our results support the concept that these effects are diminished under the conditions of this study. Factors involving duration of exposure and variables including species, dose and dose route, and competing central/peripheral effects could explain these results.

142 NEUROTOXICITY FROM OUTDOOR, CONSUMER EXPOSURE TO A METHYLENE CHLORIDE PRODUCT

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A 46 year old man was priming a fiberglass boat with a product containing 90% methylene chloride (MC). He worked outdoors, air temperature in the upper 80's F., on a calm day, in an area that was protected from wind. The subject worked underneath the boat about 70% of the time. The boat was elevated two feet above the ground, and his head was about 12–18 inches from the surface being primed. About a quart of the primer was used over the 3.5 hours of work time. MC is denser than air, and fell into the subject's face. Based on modelling, his exposure was estimated to be about 5,000 – 7,000 ppm, while the proposed OSHA standard is 25 ppm. After the bottom was primed, he began to paint the bottom with anti-fouling paint. He fell unconscious, and friends who were nearby dragged him out from under the boat. He was unresponsive for less than one minute, and then was disoriented and appeared intoxicated. He complained of a headache, which he reports to date, and was observed to be forgetful, clumsy, and with poor visual-spatial judgement. Five days later, he presented at the local hospital and was diagnosed with methylene chloride poisoning. Methylene chloride is partially metabolized to carbon monoxide, and it's toxicity is a combination of solvent toxicity (narcosis, anesthesia) and CO toxicity (carboxyhemoglobinemia). Upon multiple neuropsychological examinations by more than one examiner, the subject was found to have deficits incompatible with his prior high level of social and vocational function. He was positive on the Neurotoxicity

Screening Survey, showing symptoms consistent with neurotoxicity; estimated to have a pre-exposure IQ of 138; current performance IQ of 121; Paced Auditory Serial Addition Test <1%; and deficits in memory and emotional function. Memory consolidation was impaired; the subject forgot that he had been examined when questioned two weeks later. He was unemployable due to deficits in attention, memory and perception.

143 THE OLFACTORY SYSTEM AND THE BLOOD BRAIN BARRIER: COMPARISON OF ORAL AND NASAL ROUTES OF EXPOSURE TO LEAD (Pb) IN THE RAT

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The neurons of the olfactory system are not protected by the Blood Brain Barrier (BBB). The olfactory nerve is a direct anatomical connection between the environment and the brain and may serve as a direct route of neurotoxic exposure to the Central Nervous Systems (CNS). We Hypothesize that the olfactory pathway may transport neurotoxins, such as lead (Pb), to the brain. In comparison to the oral route of exposure, where the blood carries lead from the gut to the brain, the nasal route may bypass the protective mechanism of the BBB. Through a comparison of lead ingestion and inhalation, this study examined the presence of this environmental toxin in the CNS and investigated the role of the olfactory systems as a conduit for access to the brain. Male, Sprague Dawley rats were exposed to a single equivalent dose of 0.5 mg of lead acetate administered via oral gavage or nasal irrigation. Oral and nasal control groups were exposed to deionized distilled water or physiological saline(0.9%), respectively. Blood was sampled at 0, 2, 4, and 8 days after exposure. BPb levels(ug/dl) were determined by graphite furnace atomic absorption spectrophotometry (AAS) and indicated that there were significant differences between the oral and nasal route of exposure. Oral exposure gave rise to higher Mean BPb levels as compared to the nasal route of exposure. Mean BPb levels for the nasally exposed animals did not differ significantly from that of controls. CNS tissue was dissected on days 4 and 8 using a brain matrix. The olfactory bulbs and olfactory areas of the cerebrum (+6.70mm/-2.56mm Anterior-Posterior (AP)); remaining cortical areas and areas of the midbrain (−2.56/-8.72mm AP); and the cerebellum (−8.72/-14.60mm AP); were analyzed separately by AAS. Results showed detectable levels of Pb (ug/g) in the brains of both orally and nasally exposed animals. The data suggested that lead may enter the rat brain via the olfactory system as well as cross the BBB. (Supported by NIEHS grant #T35-ES07 298-01)

144 ENHANCED STABILITY/INCREASED LEVELS OF C-MYC mRNA AND INCREASED C-MYC PROTEIN LEVELS IN LEAD CHROMATE AND 3-METHYLCHOLANTHRENE TRANSFORMED 10T1/2 CELL LINES

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Lead chromate is carcinogenic to animals and humans. Molecular mechanisms by which lead chromate induces carcinogenesis are unknown. We tested the hypothesis that lead chromate activates oncogenes, which is partly responsible for lead chromate carcinogenesis. In C3H/10T1/2 (10T1/2) mouse embryo cells, we previously showed that lead chromate induced morphological and neoplastic transformation. In this study, we found that two lead chromate and two 3-methylcholanthrene (MCA) transformed 10T 1/2 cell lines had 7–8 fold higher steady-state levels of the 2.3 kb c-*myc* RNA transcript and 2-fold higher steady-state levels of c-*myc* protein than 10T1/2 cells without gross structural changes in the c-*myc* gene, or in c-*myc* RNA transcripts and without alterations in transcription rates of the c-*myc* gene. However, c-*myc* RNA in transformed 10T1/2 cell lines had an increased stability. RT-PCR-RFLP analysis showed no restriction polymorphisms in c-*myc* exons 1, 2, or 3 in non-transformed or in lead chromate or MCA transformed 10T1/2 cell lines. Our data suggests that constitutively higher steady-state levels of c-*myc* RNA are due to mutations/deletions not detectable by RFLP analysis in a c-*myc* gene in lead chromate or in MCA induced transformed cell lines, leading to increased stability and accumulation of c-*myc* RNA. These higher c-*myc* RNA levels likely contribute to induction/maintenance of morphological and neoplastic transformation in lead chromate and MCA induced transformed 10T1/2 cell lines. Cloning and sequencing of altered c-*myc* genes from transformed 10T1/2 cell lines is in progress. Supported by grant R01 CA40468 to J. R. Landolph.

145 GROWTH FACTOR PHOSPHOPROTEIN EXPRESSION IS ALTERED IN MUTANT P53 EXPRESSING CELL LINES

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The ability of the cell to modulate protein phosphorylation status is highly critical to the maintanance of signal transduction pathways and cell cycle regulation. Oncogenic activity can alter protein kinase function, but the downstream targets of these activities still remain to be clearly identified. To determine whether the expression of mutant p53 is capable of changing protein phosphorylation giant two-dimensional gel electrophoresis was utilized. Here we show that in four out of five mutant p53 cell lines examined [v135, M175, KH(215), R248, R273] the phosphorylation pattern of three proteins are altered as compared to a p53 null [10(3)] and a wild-type p53 cell line [MEF]. These observations have been confirmed in a second unrelated cell system that allows for the alteration of p53 status (mutant vs. wild-type) using IPTG. While protease digestion of these proteins reveal that they share common peptide cleavage patterns, the degree of phosphorylation status is varied between peptide fragments. In addition two of these proteins have been observed to be responsive to EGF, PDGF and TPA in Swiss 3T3-fibroblasts. Taken together, these findings demonstrate that select mutant p53 proteins can perhaps alter the phosphorylation status of proteins that may be pertinent to normal signal transduction events. Supported by NIH DK16177

146 DETECTION OF K-RAS GENE MUTATIONS IN NON-NEOPLASTIC LUNG TISSUE AND LUNG CANCERS

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Oncogene and tumor suppressors gene mutations are candidate biomarkers for cancer risk assessment and lesion detection. The K-ras oncogene has previously been associated with non-small cell lung cancer (NSCLC), particularly adenocarcinomas in which reported rates of mutation have approached 30–40%. We have analyzed non-malignant lung tissue from patients with lung cancer and primary lung cancers for K-ras gene mutations. Mutations were detected in 37% cancers and 23% non-malignant lung tissue from patients with cancer. The majority of tumors testing positive were adenocarcinoma of the lung. Normal DNA controls, including peripheral blood lymphocytes and normal lung from non-smokers, were negative. The ability to detect genetic alterations in non-malignant lung tissues is consistent with the concept that genetic alterations are involved in field cancerization of the aerodigestive tract. Supported by SWEHSC: NIEHS Center Grant P30-ES-O6694 and Cancer Research Foundation of America.

147 ALTERATIONS OF P53 GENE IS A FREQUENT EVENT IN UVR-INDUCED SQUAMOUS CELL CARCINOMA AND SPINDLE CELL TUMORS ON SENCAR MICE SKIN

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Abstract: An ultraviolet radiation (UVR) induced Sencar mouse skin carcinogenesis model was established to investigate the frequency of p53 gene alterations in different carcinogenetic stages, including UV-exposed skin, papillomas, squamous cell carcinomas (SCCs) and malignant spindle cell tumors (SCTs). In the expression level, p53 positive nuclear staining was found in 10/37 (27%) of SCCs and 12/24 (50%) of SCTs but not detected in normal skin nor papillomas. DNA was isolated from paraffin-embedded normal skin, UV-exposed skin and the tumor sections. P53 gene (Exon 5 and 6) was amplified from 90% (40/44) of the sections by using nested PCR. A total of 78 PCR amplified exons were applied to SSCP analysis. Sebsequent sequencing analysis showed 6 point mutations in exon 5 (3 in SCTs, 2 in SCCs and 1 in UV-exposed skin). The pattern of the mutations (3/6 are C->T transitions, and 2/6 are C->A transition) indicated that the mutagenic lesions induced by UVR. These results indicate that alteration in P53 gene is a frequent event in SCCs and later stage SCT tumors induced by UVR.

148 THE INHIBITORY EFFECT OF VARIOUS NON-GENOTOXIC HEPATOCARCINOGENS ON THE ENU-INDUCED LYMPHOMAGENESIS IN PIM-1 TRANSGENIC MICE

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To assess the carcinogenic potential of chemicals, pim-1 transgenic mice have recently been used for the bioassay. Pim-1 gene is implicated in T-cell lymphomagenesis because of its frequent activation by proviral insertion of murine leukemia virus in T-cell lymphoma. Pim-1 transgenic mice develop lymphoma within 34 weeks. The carcinogen, N-ethyl-N-nitrosourea (ENU) induces the lymphomagenesis. We examined the promotional activity of lymphomagenesis in the mice for the non-genotoxic carcinogens. Six or 8 week-old male pim-1 transgenic mice and 7 week-old C57BL mice were intraperitoneally dosed with ENU at 60 mg/kg (ENU initiation) and then fed non-genotoxic carcinogens; phenobarbital, clofibrate or d, l-ethionine for 20 weeks. ENU initiation alone and non-treatment groups were set as the controls. Histopathology for liver, spleen and lymph node, expression of pim-1 gene and mutation of p53 gene were analyzed. Contrary to our expectation, incidence of lymphoma in transgenic mice was decreased by these non-genotoxic carcinogens. Preneoplastic or neoplastic lesions were not seen in the liver. mRNAs of pim-1 gene were expressed and mutation of p53 gene was not detected in spleen of mice with lymphoma. Although the carcinogenic potential of non-genotoxic carcinogens was not assessed by using pim-1 transgenic mice, these chemicals interestingly show inhibitory effect on the lymphomagenesis in pim-1 transgenic mice.

149 TGFα TRANSGENIC MICE EXHIBIT HIGH SENSITIVITY TO TUMOR GROWTH IN MULTISTAGE SKIN CARCINOGENESIS

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Sponsor: M P Waalkes

The role of TGFα was investigated in multistage skin carcinogenesis using TGFα transgenic mice (MT42). Female MT42 and parent strain CD-1 mice were treated with topical skin application of DMBA followed by TPA once a week for 30 weeks. Multiplicity and size of skin tumors were markedly higher in MT42 mice than in CD-1 mice. The majority of tumors were papillomas, but MT42 mice also developed squamous cell carcinomas. Epidermal cell proliferation, as measured by BrdU and PCNA labeling 48hr after TPA treatment alone, was significantly higher in MT42 skin as compared to CD-1 skin. Thus, TGFα, itself a potent mitogen, appeared to enhance skin tumor promotion. Additional studies on molecular mechanisms on the enhanced tumor promotion are in progress.

150 ACTIVATION OF *RAS* ONCOGENE IN DIETHYLNITROSAMINE-INDUCED LIVER CARCINOGENESIS IN MEDAKA (*ORYZIAS LATIPES*)

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Members of the *ras* gene family are of implied biological significance in oncogenesis, and have been studied in a variety of species. To our knowledge, the sequences of ras genes in the medaka (*Oryzias latipes*) have not been reported. Using reverse transcription polymerase chain reaction (RT-PCR), TA cloning and sequencing, we have sequenced three ras genes (*ras*-1, *ras*-2, and *ras*-3) expressed in the normal medaka liver. Sequences cover codons 1 to 77, including codons 12, 13 and 61, sites where oncogenic mutations are most frequently observed in various tumor types. In this report, ras point mutation activation was investigated in diethylnitrosamine (DEN) -induced medaka liver tumors. Using PCR and allele specific oligonucleotide (ASO) probing, 3 of the 11 DEN-induced medaka liver tumors had evidence of activating point mutations at codon 12 (GGT to GAT transition) of medaka *ras*-2. Using RT-PCR and sequencing directly, another DEN-induced medaka liver tumor showed the same mutation. This is the first report of the role of *ras* oncogenes in DEN-induced medaka liver tumors and results in medaka indicate that *ras* mutation play a role similar to that observed in mammalian hepatic carcinogenesis.

151 INDIVIDUAL VARIATION IN p53 AND Cip1 PROTEIN EXPRESSION FOLLOWING α-PARTICLE IRRADIATION

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Alpha particles from radon progeny are thought to cause 10% of lung cancers. The cellular response to alpha particle damage includes increased expression of p53 and Cip1 proteins. p53 and Cip1 cause a cell cycle arrest in G_1 that provides increased opportunity for DNA repair prior to replication. Individual variations in the p53 response to alpha particles may represent variations in radon-related lung cancer susceptibility. The p53 response was examined in secondary passage fibroblast cultures obtained from nine neonatal foreskin samples (surgical waste). The resulting fibroblast lines were grown on 1.5 μm Mylar® and were exposed to α-particles from an electroplated ^{239}Pu source. Three populations were identified as having statistically different mean percentages of p53-positive cells (ANOVA $P<0.05$), and were labeled as low ($\bar{x}$ = 1% ± 3.4%), normal ($\bar{x}$ = 11.8% ± 4.6%), and high responders ($\bar{x}$ = 27.3% ± 1%). Each of the fibroblast lines was then exposed to graded doses of α -particles and harvested 6 h after exposure. Immunostaining for p53 revealed a positive dose-response relationship in each of the normal responder cell lines. The high responder cell line demonstrated a relatively sensitive p53 response and the low responder cell lines demonstrated relatively insensitive p53 responses. Cip1-immunostaining of the above cell lines revealed that the high responder line and the low responder lines did not exceed 10% Cip1 + cells at 2 Gy while normal responders averaged 52%. No significant differences in clonogenic survival were observed among the low, normal, or high responder cell lines. These findings suggest significant variations exist in the p53-regulated response to DNA damage from alpha particle radiation that may contribute to radon-caused lung cancer susceptibility. Research sponsored by the Office of Health and Environmental Research, U. S. Department of Energy, under Contract No. DE-AC04,76EV01013.

152 EVIDENCE FOR GENE COOPERATION IN 7, 12-DIMETHYLBENZ[A]ANTHRACENE (DMBA) TWO HIT PROTOCOL IN FVB/N MOUSE SKIN

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Recently, we demonstrated that there exist unidentified non-Ha-*ras* DMBA target genes that can cooperate with mutant v-Ha-*ras* to give rise to increased papilloma frequency, growth, and malignant conversion in mutant v-Ha-*ras* transgenic mouse skin. In order to determine if unidentified non-Ha-*ras* DMBA target genes exist that could cooperate with mutant Ha-*ras* to increase papilloma frequency, growth, or malignant conversion in nontransgenic mouse skin, we employed a DMBA two hit treatment protocol in FVB/N mouse skin. DMBA-initiated/12-O-tetradecanoylphorbol-13-acetate (TPA)-promoted mice received a second dose of DMBA 9 weeks after the start of promotion but prior to tumor development. DMBA-initiated/TPA-promoted (D/T) and DMBA-initiated/TPA-promoted/DMBA-initiated/TPA-promoted (D/T/D/T) FVB/N mice produced similar papilloma yields, incidence, and growth rates. However, D/T/D/T FVB/N mice exhibited a six week decrease in malignancy latency and marked increases in malignancy frequency and incidence. Papillomas, squamous cell carcinomas, and fibrosarcomas arising from both treatment protocols were found to contain the characteristic DMBA-induced A^{182}->T mutation in c-Ha-*ras*. These results indicate that unidentified non-Ha-*ras* DMBA target genes exist that can cooperate with mutant Ha-*ras* to decrease malignancy latency and increase malignancy frequency and incidence in FVB/N mouse skin.

153 PARENTAL ALLELE BIAS IN ACTIVATION OF K-*RAS* IN AFLATOXIN B_1 (AFB_1)-INDUCED LUNG TUMORS OF AC3F1 (A/J × C3H/HeJ) MICE

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Point mutation at codon 12 of the K-*ras* protooncogene is often implicated in chemically-induced mouse lung tumors and in human lung adenocarcinomas. There is also evidence to suggest that K-*ras* is linked to mouse strain differences in lung tumor susceptibility. In this study, 65 female AC3F1 mice were treated with AFB_1 (150 mg/kg i.p. divided into 24 doses over 8 weeks), and 15 months after dosing, mice were killed and tumors were collected. Of the

76 adenomas and adenocarcinomas analyzed by single strand conformation polymorphism (SSCP) and direct sequencing, 75 possessed codon 12 mutations (46 GTT; 14 GAT; 13 TGT; 2 TTT - Normal = GGT), and one had a GGC to CGC mutation in codon 13. Allele-specific PCR followed by SSCP analysis revealed that 73 of the 76 observed K-*ras* mutations originated from the A/J (lung tumor susceptible) parent. These findings are consistent with K-*ras* mutation at guanine residues being an early event in AFB_1-induced pulmonary carcinogenesis, and support an association between parental bias for K-*ras* mutation and differences in mouse lung tumor susceptibility. (Supported by Medical Research Council of Canada Grant #MT10382).

154 TIME- AND CARCINOGEN-DEPENDENT INDUCTION OF ALTERED HEPATIC FOCI IN ALBUMIN.SV40 TRANSGENIC RATS

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Transgenic rats containing the albumin.SV40 construct develop hepatic neoplasms with 100% incidence by 6–8 months of age. Female Sprague-Dawley rats were mated with male albumin.SV40 rats and the progeny were used in the time course and dosing experiments. Half of each litter express the transgene (TG^+), while the other half are negative for the transgene. Rats were sacrificed at 3, 12, or 24 weeks of age. Alternatively, rats were dosed with diethylni-trosamine (3, 10, 30 mg DEN/kg), aflatoxin (0.05, 0.2 mg AFB1/kg), or irradiation (300 RAD) at 5 days of age and sacrificed at 12 weeks of age. At sacrifice, sections were fixed with formalin for pathological diagnosis. Additional sections were frozen and serial sections were stained for placental glutathione S-transferase (GST), T Antigen (T Ag) and p53 expression. A time-dependent induction of T Ag expressing lesions was detected in TG^+ rats. Female rats contained a greater percentage of the liver occupied by T Ag^+ lesions than males, which may be due to an enhanced expression of insulin growth factor 2 in female but not male TG^+ rats. A marked increase in T Ag^+ lesions was induced by administration of 10 and 30 mg DEN/kg and by 0.2 mg AFB1/kg. An increased incidence of malignancy was also observed in TG^+ rats treated with the highest dose of DEN. The increase in malignancy with DEN, but not AFB1 or RAD may indicate that clonal induction of neoplasms in animals with a genetic predisposition to cancer is only sensitive to selected carcinogens. Supported by CA-22484 and -45700 from NCI.

155 CHARACTERIZATION OF THE PIM TRANSGENIC MOUSE AS A MODEL SYSTEM FOR THE STUDY OF LYMPHOMA ETIOLOGY AND PREVENTION

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The use of transgenic animals in toxicology and carcinogenesis research has increased in attempts to develop animal models to study mechanisms of toxicity and disease etiology, and to evaluate specific agents for chemopreventive activity. The integration of chemical carcinogenesis techniques with transgenic animal technology has provided a transgenic animal model, the PIM mouse, in which lymphoid neoplasia can be induced rapidly, and in high incidence (Breuer *et al.*, 1989, 1991). PIM mice overexpress the *pim*-1 oncogene, and are highly susceptible to lymphoma induction by a single dose of the carcinogenic nitrosamide, N-ethyl-N-nitrosourea (ENU). Examination of the dose- and time-response characteristics of tumor-associated mortality in PIM mice following a single i.p. injection of ENU at doses ranging from 25 to 200 mg/kg body weight showed that mortality occurred in a dose-dependent manner and followed a predictable time course. Relatively little mortality associated with lymphoma induction was seen in the first 10 weeks after ENU administration. Between approximately 10 and 20 weeks, a nearly linear decrease in survival was seen in all dose groups; survival was inversely related to dose. A plateau in survival was reached approximately 20 weeks after ENU administration, and little additional mortality was seen after this period. Essentially 100% of mortality in ENU-treated PIM mice was associated with the development of metastatic lymphoma. The tumors appeared to originate in the thymus, with spread to the spleen and peripheral lymph nodes, resulting in gross enlargement of these tissues. Microscopically, the tumors were characterized by a diffuse proliferation of lymphoblastic cells with obliteration of normal architecture. Metastases were commonly found in the liver, lung and kidneys of affected animals. Results indicate that the PIM transgenic mouse may provide a useful model for the detection of hematopoietic carcinogens or evaluation of agents as inhibitors of lymphomagenesis.

156 MAGNETIC FIELD EXPOSURE AND RISK OF LYMPHOMA IN PIM AND TSG-P53 TRANSGENIC MICE

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Epidemiologic data suggest that exposure to 60 Hz magnetic fields [EMF] may increase the risk of hematopoietic neoplasia in humans. However, the EMF epidemiology data base is inconsistent, and increases in relative risk reported for populations exposed to EMF are small. Because epidemiology does not now support conclusive risk assessments for EMF, well-controlled animal studies may prove crucial to identification of its potential hazards. To evaluate its possible activity as a promoter of hematopoietic neoplasia, the effects of EMF on lymphoma induction were determined in two strains of mice that are genetically predisposed to the disease. PIM mice, which carry the *pim*-1 oncogene, are highly sensitive to lymphoma induced by *N*-ethyl-*N*-nitrosourea [ENU]; ENU-treated PIM mice were studied as a "high incidence" lymphoma model system. TSG-p53 mice, in which the p53 tumor suppressor gene has been deleted, develop lymphoma spontaneously as an age-related change; hemizygous TSG-p53 mice were studied as a "low incidence" lymphoma model. Beginning one day after a single i.p. injection of 25 mg ENU/kg body weight, groups of 30 PIM mice/sex were exposed for 18.5 hrs/day to EMF at field strengths of 0 G (control), 0.02 G, 2 G, or 10 G. An additional group of 30 PIM mice/sex were exposed intermittently (1 hr on, 1 hr off) 10 G fields. Groups of 30 TSG-p53 mice per sex were exposed continuously to EMF at field strengths of 0 G or 10 G; TSG-p53 mice received no ENU. Studies were terminated after 175 days of EMF exposure, and lymphoid tissues were evaluated histopathologically. When compared to strain- and sex-matched controls, EMF neither increased lymphoma incidence nor decreased lymphoma latency in either model system. Tumor-related mortality was similar in all groups within a strain. These results demonstrate that EMF does not stimulate the development of lymphoid neoplasia in either PIM or TSG-p53 mice. Our data do not support the hypothesis that EMF is a significant risk factor for hematopoietic neoplasia in humans. (NIH-NIEHS-NO1-ES-25351)

157 REGULATION OF A STRESS-ACTIVATED PROTEIN KINASE BY A NOVEL TUMOR PROMOTER

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Previous studies have suggested that tumor promoters contribute to carcinogenesis by modulating the activity of protein kinases involved in signal transduction. In this study, we show that palytoxin, a non-TPA-type skin tumor promoter, induces a signaling pathway leading to the activation of stress-activated protein kinase/c-Jun N-terminal kinase (JNK) in Swiss 3T3 fibroblasts. Palytoxin is a potent activator of JNK. In contrast to epidermal growth factor, which causes a transient activation of JNK in Swiss 3T3 cells, palytoxin causes prolonged enzyme activation. Since stimulation of ion flux appears to play an important role in the mechanism of action of palytoxin in other systems, we investigated the role of calcium and sodium influx in the activation of JNK. Our results indicate palytoxin induces the activation of JNK through a sodium-dependent signaling pathway that does not require a rise in intracellular calcium. In contrast to palytoxin, the tumor promoters phorbol 12, 13-dibutyrate, thapsigargin, and okadaic acid do not activate JNK in this system. Finally, palytoxin, unlike the phorbol ester tumor promoters, does not activate the p42/p44 mitogen-activated protein kinases. Our results demonstrate that palytoxin can activate a protein kinase cascade that is distinct from the protein kinase cascades activated by the prototypical phorbol ester tumor promoters and other potent skin tumor promoters.

158 BENZO(a)PYRENE-INDUCED MUTAGENESIS OF p53 HOTSPOT CODONS 248 AND 249 IN HUMAN HEPATOCYTES

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Human tobacco-related cancers show a high frequency of G to T transversions in several mutation hot spot regions of the p53 tumor suppressor gene, likely the result of specific mutagens in tobacco smoke, most notably benzo(a)-pyrene.

In order to gain insight into the mechanism of formation of these G to T

transversions in tobacco-associated carcinogenesis we studied the mutagenesis of codons 247 to 250 of p53 by benzo(a)pyrene in human hepatocellular carcinoma cells by restriction fragment-length polymorphism-polymerase chain reaction (RFLP-PCR) genotypic analysis. Benzo(a)pyrene preferentially induced transversions of G to T in the second and third position of codon 248 and C to A in the first position of codon 248. However, benzo(a)pyrene did not induce base-pair changes in codon 249 which is a mutational hotspot in aflatoxin-related hepatocarcinogenesis, where predominantly a G to T transversion in the third position of codon 249 is observed. The benzo(a)pyrene-induced G to T transversion in the middle position of codon 248, where arginine is changed into leucine, is frequently observed in tumors of the lung. The other two benzo(a)pyrene-induced base-pair changes in codon 248, namely the transversions of C to A in the first position and G to T in the third position, do not lead to a change in the amino acid composition of the p53 protein. These mutations remain silent and are therefore not selected in a tumor. It follows that benzo(a)pyrene-induced mutability on the DNA level in p53 codons 247 to 250 correlates well with the type of mutation found in tumors of the lung. Therefore, our results are in agreement with benzo(a)-pyrene as etiological agent in tobacco-related cancers.

159 METHYLATION STATUS OF THE MYC ONCOGENE IN B6C3F1, C3H/He and C57BL/6 MICE

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Methylation of DNA (5-methylcytosine content of DNA) is an important factor regulating gene expression. We are investigating the role that alterations in DNA methylation play in the abnormal gene expression involved in carcinogenesis, and have emphasized the hypothesis that hypomethylation is a nongenotoxic mechanism underlying tumor promotion. The protein encoded by the myc gene is a transcription factor that plays a role in regulating cell proliferation and apoptosis. Inhibition of myc expression can inhibit carcinogenesis. Southern analysis following restriction with a methylation-sensitive endonuclease (e.g., MspI, HpaII, HhaI, XhoI), was employed to assess the methylation status of myc in hepatic DNA isolated from the tumor-prone B6C3F1 mouse (C57BL/6♀ X C3H/He♂), and its sensitive paternal and relatively resistant maternal strains. Two probes were used: 1) A c-myc probe which is complementary to a region in the third exon; and 2) a v-myc probe which is complementary to portions of all three exons of myc. In this manner, we have examined the methylation status of a broad segment of the gene and discerned changes in the 3′ as compared to the 5′ portion. Our results indicate that myc is methylated in mouse liver, and no differences were detected between the three strains. Placement of B6C3F1 and C57BL/6 mice on a choline devoid, methionine deficient (CMD) diet for 12 weeks, a treatment known to cause liver tumors in rodents, results in hypomethylation at a HhaI site(s) in both strains. The fact that myc is methylated in mouse liver DNA and placement of mice on a CMD diet results in decreased methylation together indicate that hypomethylation of this gene could facilitate its aberrant expression that appears to play a role in carcinogenesis. Supported by NIH NIEHS ES05299, and Intl. Life Sci Inst, HESI.

160 IDENTIFICATION OF MOLECULAR TARGETS OF CARCINOGENESIS IN MOUSE LIVER TUMOR FORMATION

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The B6C3F1 mouse is used extensively in many cancer bioassay programs, including those conducted by the National Toxicology Program. Although liver tumor induction is the most common end point, little is known about the molecular changes involved in the formation of these tumors. We designed a two-year study to provide the tissues required to determine the cellular and molecular changes associated with mouse liver tumor formation. Groups of mice were untreated or treated with vinyl carbamate, phenobarbital (PB), or dioxin (TCDD). Necropsy time points were selected to assess adenoma and carcinoma development. At 9 months post treatment, all the vinyl carbamate-treated mice had visible masses with a tumor multiplicity (TM) of 6 tumors per tumor- bearing animal. At the same time point, 20% of the TCDD- treated mice exhibited masses with a TM of 1. At 1 year post treatment, TCDD-dosed mice had a 63% incidence of tumor formation with a TM of 1.8. Of the untreated and PB-treated mice 12% and 56%, respectively, had visible masses one year post treatment. At 16 months, 96% of the TCDD- and PB-treated mice had tumors, while untreated mice had a 38% tumor incidence (TM 6.2, 7.9, and 1.7, respectively). Spontaneous hepatocellular carcinomas from mice showed gene alterations in IGF-II, c-myc, cyclin D1, and EGFR. These studies demonstrate differences in time to tumor induction and multiplicity with both genotoxic and nongenotoxic agents. The identified alterations in growth regulatory genes may represent molecular targets important in mouse hepatocarcinogenesis.

161 IDENTIFICATION OF NEW GENES INVOLVED IN THE PROCESS OF NON-GENOTOXIC CARCINOGENESIS

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A variety of non-genotoxic chemicals (peroxisome proliferators, enzyme inducers) elicit hepatocellular tumours when administered to rats and mice over long periods. The basic mechanism(s) by which these compounds induce tumours in these rodents and not in humans is unknown however the stimulation of cell proliferation appears to be necessary. Using molecular approaches the identification and subsequent characterisation of novel genes induced only by non-genotoxic carcinogens will provide new information on these unknown mechanisms. We are identifying and isolating novel genes expressed in proliferating liver cells induced by non-genotoxic carcinogens but not by partial hepatectomy using the technique of differential display (RNA display or DD-PCR). Rats have been treated with one of three non-genotoxic chemicals (PCN, PB and the peroxisome proliferator MCP), or two levels of partial hepatectomy (34% and 68%) over seven timepoints. Northern blot analysis shows that expression of early response genes, such as *c-fos* and *c-jun*, are found only in the regenerating tissue confirming earlier evidence that proliferation might be controlled by different regulatory pathways. We have shown, by BrDU incorporation and S-phase gene expression, that each treatment induces a different S-phase response. From these findings we chose treatment material with similar S-phase and early gene expression levels for differential display. Many reproducible display patterns have been produced using a wide range of primer sets from which differentially expressed genes have been isolated and cloned. Northern and sequence analysis suggests some of these to be interesting suggesting a possible role in non-genotoxic carcinogenesis.

162 LUNG CARCINOGENESIS OF 4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANONE (NNK) AND URETHANE IN C-HA-RAS TRANSGENIC MICE

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We have shown previously that the transgenic mice carrying human hybrid c-Ha-ras gene were very sensitive to urethane and 6-nitrochrysene, lung carcinogens. Some of the induced tumors had point mutations at codon 61 of c-Ha-ras gene. Therefore, NNK, a tobacco specific nitrosamine, and urethane were investigated using transgenic mice. Female transgenic mice (TG mice) and litter-mate non-transgenic mice (non-TG mice) were given 10μmol NNK or saline ip. Additional groups received 500ppm urethane in drinking water during weeks 25 to 27. All animals were sacrificed at week 44. Neoplastic lesions were observed mainly in the lungs. NNK showed weak carcinogenesis in both TG and non-TG mice. Urethane strongly enhanced lung tumor incidence in TG mice, but not in non-TG mice. The study of gene mutations in the induced lung tumors are currently being investigated. The present results indicate that these transgenic mice may be useful for the detection of lung carcinogens and for the study of lung neoplasia development.

163 P53 MUTATIONAL ANALYSIS IN PROGRESSIVE GROWTH OF RAT BLADDER CANCERS INDUCED BY BBN

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We have examined the relationship of p53 mutations to progression of rat bladder cancers. F344 male rats were given 500ppm *N*-butyl-*N*-(4-hydroxybutyl) nitrosoamine (BBN) in drinking water during the first 10 weeks. Ten weeks later, they were given an ip injection of 50mg/kg bw *N*-methyl-*N*-nitrosourea (MNU), and treated with indomethacin (20ppm) in the drinking water for 20 weeks, alone or in combination. The experiment was terminated at week 40. All bladders were examined histologically, and for p53 mutations. At week 40, carcinomas were observed in 80~100% of all groups. In group of BBN + MNU, highly invasive bladder cancers were found, and p53

mutations were detected with low frequency. However, no relation to invasiveness was observed. No modification by indomethacin was noticed. These results indicate that p53 mutation is not a critical event in tumor progression of rat bladder.

164 CHARACTERIZATION OF THE RAT NEUROFIBROMATOSIS (NF 2) GENE AND ITS INVOLVEMENT IN MESOTHELIOMA

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The NF 2 tumor suppressor gene has recently been implicated in the genesis of human mesothelioma. To investigate the role of this tumor suppressor gene in fiber-induced mesothelioma, the rat homologue of this gene was characterized and rat chrysotile-induced primary mesotheliomas and cell lines derived from chrysotile- and crocidolite-induced rat mesotheliomas were examined for NF-2 alterations. The 1792 bp coding sequence of the rat NF-2 gene has 90% nucleotide homology with the human gene and is alternatively spliced and expressed as 3.5 and 2.7 kb transcripts in normal rat tissues. In contrast, in rat mesotheliomas, the NF-2 gene is expressed as a 4.4 kb transcript. No structural alterations of this gene were detected at the level of Southern analysis, suggesting that alterations in transcription or splicing of the NF-2 gene were occurring in the transformed cells. RT-PCR to examine splicing of NF-2 exons in mesothelial cells indicated that exon splicing patterns were similar in both normal and neoplastic cells. To determine if mutations had occurred in the NF-2 coding region, SSCP and direct sequencing were used to screen 5 cell lines and 10 primary tumors for mutations. No DNA sequence alterations were observed in any of the rat mesothelioma samples. This contrasts with data reported previously for human NF-2, in which this gene was mutated in 40% of human mesotheliomas. Taken together, these data suggest that the mechanism for NF-2 involvement in the development of rodent fiber-induced mesothelioma differs significantly from that of the human disease.

165 MURINE TOXICOLOGY AND PHARMACOLOGY OF UAB-8, A CONFORMATIONALLY CONSTRAINED ANALOG OF RETINOIC ACID

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(2*E*,4*E*,6*E*)-8-[3′-Ethyl-2′-(1-methylethyl)-2′-cyclohexen-1′-ylidene]-3,7-dimethyl-2,4,6-octatrienoic acid (UAB-8) has potent activity in preventing papillomas on the skin of mice similar to the homolog containing one less carbon atom (J. Med. Chem. *37*:4499, 1994). To determine the toxicological profile for UAB-8, relative to all-*trans*-retinoic acid (RA), female CD-1 mice were randomized into groups of 8. There were 3 experimental groups (dosed with UAB-8 in corn oil), 3 reference groups (dosed with RA in corn oil), and 1 vehicle group (dosed with corn oil). For 29 days, the doses (0.05, 0.1, and 0.2 mmol/kg/day) were administered p.o. The low dose of RA caused a 25.5% decrease in weight gain; the low dose of UAB-8 caused an 8.8% decrease. For higher doses of RA and UAB-8, the effects on body weights were similar. Relative to those that received RA, mice dosed with UAB-8 had a decreased incidence of clinical signs of toxicity. As determined at necropsy, fractures, skin abnormalities, and splenomegaly were observed in some mice dosed with RA but not in any dosed with UAB-8. Histopathologic findings including lymph node hyperplasia in some of the mice dosed with RA but none in those dosed with UAB-8. All doses of RA produced microscopic lesions in the bones of mice; only the highest dose of UAB-8 had this effect. For mice dosed i.v., there was a saturated phase of elimination of RA from plasma; no such phase was noted for UAB-8. For this compound, values for $t_{1/2\alpha}$ and $t_{1/2\beta}$ were 0.47 and 17.1 hr, respectively. Relative to RA, UAB-8 has a favorable toxicological profile and different pharmacokinetics. (Supported by PO1 CA34968 from NCI, NIH, DHHS)

166 DYNAMICS OF RENAL EXCRETION OF PHENOL AND MAJOR METABOLITES IN THE URINE OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Renal excretion of phenol (PH), phenylglucuronide (PG), phenylsulfate (PS), and hydroquinone (HQ) were determined in 700–800 g rainbow trout continuously exposed (144 h) to PH (5mg/L) in respirometer-metabolism chambers. The plasma uptake time course was obtained from the dorsal aorta, while the urine time course was acquired by a bladder catheter. PH and HQ in plasma were at steady-state in 4 h. PG levels in both plasma and urine increased throughout the exposure. PS in plasma increased for 144 h, while urine PS plateaued near 24 h. This indicated saturation of sulfate conjugation at a plasma concentration of 6mg/L. Of the net PH uptake at steady-state (144 h), 55% was eliminated in the urine (PG = 78%, PS = 19%, PH = 3%) and the rest extrarenally. Renal clearance rates (C_x) calculated for PH, PG, and PS were 4.40, 8.28, and 20.50 ml/kg/hr, respectively. Urine clearance ratios (C_x/GFR) were 0.74, 1.49, and 3.86 for PH, PG, and PS, respectively. These results support tubular transport in fish and a dose dependence for the sulfate conjugation system.

167 BINDING OF ACRYLONITRILE TO PARVALBUMIN

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A previous study has shown that acrylonitrile (ACN) has a long half-life in rainbow trout muscle and that ^{14}C-ACN appears to be bound to a 10,000 dalton protein in muscle. The labeled protein was purified from muscle of trout exposed to ^{14}C-ACN, separated on 20% SDS-PAGE and digested for amino acid analysis and sequence analysis. These studies indicated that the labeled protein was the Ca++ binding protein parvalbumin. Parvalbumin is an important calcium binding protein thought to be involved in the regulation of calcium levels in various parts of the body ranging from neurons to fast twitch muscle contractions.

In order to study the reaction between parvalbumin and ^{14}C-ACN, frog parvalbumin was incubated with ^{14}C-ACN *in vitro* under various conditions. These studies indicated that the maximum labeling occurred at 1 nmole/nmole parvalbumin and at pH7. Amino acid analysis of the labeled protein indicated that the labeled amino acid was probably histidine, and endoproteinase Glu-C (V8) digestion studies revealed that the ^{14}C was in the 1-81 amino acid segment of the protein, an area that contains 2 histidines. Supported by CYTEC, Inc. West Paterson, NJ.

168 CHARACTERIZATION OF THE PROPERTIES OF COCAINE IN BLOOD: CLEARANCE, BLOOD TO PLASMA RATIO AND PLASMA PROTEIN BINDING

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As part of a study to examine cocaine disposition and interaction with ethanol, it was necessary to characterize various properties of cocaine in the blood of the experimental animal. All studies were conducted using blood from healthy adult male Sprague-Dawly rats. Cocaine was incubated in whole blood at 37°C at concentrations of 500 to 4000 ng/ml. The apparent first-order rate constant for cocaine loss was independent of concentration. Blood clearance, calculated assuming blood volume to be 60 ml/kg, was estimated to be 0.056 ml/min.kg; a value considerably smaller than estimates of systemic clearance. The addition of NaF increased the rate of loss to form benzoylecgonine, as a result of increased chemical degradation and as a consequence of increased pH (to pH 8.0 over 30 hr). Ethanol had no influence on cocaine degradation and there was no evidence of cocaethylene formation.

The blood to plasma ratio of cocaine was constant (0.94–1.05) and independent of concentration (400–2000 ng/ml). This ratio was not influenced by NaF or ethanol.

The unbound fraction of cocaine determined in spiked plasma varied from 0.56 to 0.66 over the concentration range (50–2000 ng/ml). Ethanol had no effect on binding. Plasma protein binding determined from authentic blood samples taken from rats dosed iv (5–10 mg/kg) ranged from 0.40 to 0.51 (upto concentrations of 900 ng/ml). Cocaine plasma protein binding was independent of concentration but appeared to be greater in authentic ("*in vivo*") samples.

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169 PHARMACOKINETICS OF DIETHANOLAMINE IN SPRAGUE-DAWLEY RATS FOLLOWING DERMAL OR INTRAVENOUS ADMINISTRATION

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Diethanolamine (DEA) is a major industrial chemical having low acute toxicity when administered dermally. Repeated dose, dermal toxicity studies

of DEA have shown microcytic anemia and histopathological changes in the kidney as the most sensitive target tissues in rats, but dermal developmental toxicity studies of DEA in rats have been clearly negative. The present work studied the pharmacokinetics of ^{14}C-DEA in rats following single and repeated dermal administration at a dose level of 1500 mg/kg and single intravenous administration at two dose levels (10 and 100 mg/kg). DEA was slowly and poorly absorbed following a single dermal application, but a significantly greater fraction of the applied dose was absorbed following repeated dermal applications (about 21 and 42% of the applied dose following 2 days or 5 days repeated dosing, respectively). DEA was excreted principally as parent compound via the urine following administration by either route.
For internal tissues, the concentrations of radioactivity were highest in the kidneys and liver, regardless of the route of administration. Following intravenous dosing, the concentration of radioactivity was observed to gradually increase in the blood cells from 6 to 96 hours post-dosing. The significant absorption of DEA following repeated dermal application to rats indicates that the absence of developmental toxicity following dermal application was not due to a lack of DEA bioavailability.

Supported by the Chemical Manufacturers Association Alkanolamines Panel.

170 ROLE OF ENTEROHEPATIC CIRCULATION ON KINETICS OF CHLORAL HYDRATE METABOLITES IN HUMANS AND RATS

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Chloral hydrate (CH) is a by-product of drinking water chlorination, a short lived intermediate metabolite of trichloroethylene (TRI), as well as being an approved sedative/hypnotic drug. This study was undertaken to gather pharmacokinetic data on the disposition of CH for use in the development of PBPK models for both CH and TRI. CH and two of its metabolites, trichloroacetate (TCA) and dichloroacetate (DCA), have been shown to be hepatocarcinogenic in mice. The secretion of trichloroethanol-glucuronide (TCE-gluc) into bile is well established. However, the quantitative information required for determining how important this pathway is to the maintenance of plasma concentrations of TCA is not available. Rats were infused with CH at 12, 48, and 192 mg/kg and the bile, blood, urine and feces collected and analyzed for CH and its metabolites TCE, TCE-gluc, DCA, and TCA. Approximately 30% of TCA in the blood of rats is attributable to enterohepatic circulation(EHC) of TCE-gluc. CH was administered to human volunteers in 500 or 1500 mg doses, the blood and urine sampled over a 7 day period, and the specimens analyzed for CH and its metabolites. A clear distinction could be made between TCA derived from the direct conversion of the CH dose and the TCA derived from the EHC of TCE-gluc. DCA was below detection limits in the blood of both species. Pharmacokinetic constants were calculated for both species and input into the models. (This research supported by EPA # CR 822577)

171 TISSUE DISTRIBUTION OF TRICHLOROACETATE IN B6C3F1 MICE AND FISHER 344 RATS

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Trichloroacetate (TCA) has been chosen as a test chemical for the development of a pharmacokinetic (PBPK) model for water soluble compounds. TCA is formed from the metabolism of some industrial solvents such as trichloroethylene and tetrachloroethylene. It is also a byproduct of water chlorination. Mice dosed with TCA have developed hepatocellular carcinomas while rats have not. It has been shown to cause developmental malformations in rats. Male B6C3F1 mice received a continuous 1 μL/hr dose of 0.03 and 0.3 mg/μL TCA in saline via subcutaneous mini-osmotic pumps. Blood and tissues were taken at 5 days, and TCA concentration was quantified by GC after derivitization. Tissue/blood partitions were determined for several tissues. Liver, muscle and GI/ blood partitions were 0.22, 0.07 and 0.12 for the low dose, respectively. Male F-344 rats were dosed with 10 mg/kg TCA in saline by i.v. injection in the lateral tail vein. Blood and tissues were collected at 0.5, 1, 3, 6, 9, 24 and 48 hr. Total radiolabel was determined in oxidized tissues by scintillation counting. For kidney, lung, skin, testes, muscle, brain and fat the % radiolabel (tissue/plasma) remained fairly constant from the 1 hr to the 24 hr time point. Liver, heart, intestine and stomach % radiolabel increased from 1 hr to 24 hr. There was a decrease in % radiolabel at 24 hr for RBC. To determine the amount of radiolabel that was parent TCA, tissue proteins were precipitated with acetonitrile, and the soluble layer was analyzed by HPLC. A significant fraction of the radiolabel was in the precipitate for liver, indicating the possibility of binding. This description of the tissue distribution of TCA can be used in the development of PBPK model for water soluble compounds.

172 THE ELECTROCARDIOGRAPHIC AND PHARMACOKINETIC EFFECTS OF A BISPHOSPHONATE DRUG ALTERED BY A CYTOCHROME P-450 INHIBITOR IN CONSCIOUS RATS

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In an iv rat infusion study of U-91502 [3-(1, 6-Dihydro-1-methyl-6-oxo-4-phenyl-2-pyrimidinyl)-propylidene] bisphosphonic acid, tetraethyl ester)], the plasma drug concentration (PDC) profile leading to breathing rate (BR) depression suggested the occurrence of metabolic saturation. Thus, the contribution of the parent drug and/or its metabolites to its adverse effects was evaluated. Two groups of 8 rats each were pretreated with a bolus iv injection of saline (5 mL/kg) or a cytochrome P-450 inhibitor, 1-aminobenzotriazole (ABT; 50 mg/kg). At 60 min post injection, the rats were infused with ≈1 mg/kg/min of U-91502. The BR, heart rate (HR), durations of PQ, QRS, and QTc, PDCs and terminal hepatic nonprotein sulfhydril content (HNPSH) were determined. The results indicated that ABT pretreatment (1) reduced by 61% the U-91502 dose needed to develop critical BR and HR depressions and QTc prolongation; (2) elevated the PDC by 25% at the time of critical BR depression and the C_{max} by 100%; (3) shortened the T_{max} by 23%; (4) reduced the total clearance of U-91502 by 88%; (5) extended the terminal half life to almost infinity; (6) expanded the area under the PDC-time curves by 363%; (7) maintained the 180 min post infusion PDC at a level 460% higher than the saline control; and (8) did not affect the HNPSH of the 4 ABT plus U-91502 rats sacrificed immediately post infusion, but unexplainably affected the 4 rats sacrificed 180 min post infusion. In conclusion, the iv toxicity of U-91502 was due to the parent drug as indicated by a faster PDC build up and the prolonged BR and HR depressions when the metabolism and the clearance of U-91502 was inhibited by ABT.

173 COCAINE AND COCAETHYLENE KINETICS IN THE ISOLATED RAT PERFUSED LIVER: EFFECT OF DOSE, CONTINUOUS INFUSION AND ETHANOL

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Cocaine and ethanol coabuse and the production of an active metabolite (cocaethylene) has resulted in the need to fully understand the underlying mechanisms of this important interaction. The extraction ratios of cocaine and cocaethylene were measured in the single pass perfused rat (Sprague Dawley) liver model in the presence and absence of ethanol. At input cocaine concentrations of 1.65, 6.6, 16.5, 33, and 49.5 μM, and a constant input flow of 25 ml/min, the steady state extraction ratios were calculated to be 0.77, 0.77, 0.64, 0.47, and 0.25, respectively. A steady state extraction ratio of 0.62 was calculated for a constant input concentration of 6.3 μM cocaethylene. The influence of ethanol on the extraction ratio of cocaine and cocaethylene was estimated as a function of ethanol dose. At a constant cocaine input concentration of 3.3 μM and ethanol concentrations of 5, 10, and 30, mM, the cocaine extraction ratios were 0.79, 0.90, and 0.84, respectively. At a constant cocaine input concentration of 6.6 μM and ethanol concentrations of 30 and 50 mM the cocaine extraction ratios were 0.72 and 0.82. At a constant cocaethylene input concentration of 6.3 μM and an ethanol concentration of 30 mM the cocaethylene extraction ratio was 0.51. Based on these experiments, the extraction ratio of cocaine appears to be dose-dependent and does not seem to be effected by the presence of ethanol. The extraction ratio of cocaethylene in the presence of ethanol is decreased, however, the effect is small and further investigations are necessary to study this interaction. Experiments to study the effect of metabolites of cocaine on the extraction ratio are being performed. These studies should provide a clearer understanding of this drug-drug interaction.

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174 DIFFERENCES IN NEUROBEHAVIORAL EFFECTS OF THE ENANTIOMERS OF αPHENYL-2-PYRIDINE-ETHANAMINE. 2HCI IN RATS: ARL 15896AR (S) AND ARL 15895AR (R)

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The stereoisomers, ARL 15896AR (S) and ARL 15895AR (R), are NMDA receptor antagonists with the (S) enantiomer being a more potent receptor inhibitor *in vitro* than the (R) enantiomer. A rising-dose toxicity study was conducted to determine if neurobehavioral differences also existed between these enantiomers when given intravenously as a bolus to rats (4/sex). Each rising dose was given daily for ≥3 days and progressively increased (or decreased) to obtain a maximum repeatable dose (MRD). Rising doses were given over a 4 week period while the MRD was administered during the last 2 weeks of the study. Clinical signs, functional observations, and motor activity were evaluated throughout the study. Dose-related transient effects were observed immediately postdose with both enantiomers and included increased motor activity, vocalization, motor impairment, stereotypic behavior, mydriasis, tremors/convulsions, prostration, salivation, and wet-dog shakes. In contrast to the inhibitory potency at the NMDA receptor *in vitro*, the effects observed with the (R) enantiomer were more severe than those of the (S) enantiomer at equivalent doses. The MRD was estimated at 9 mg/kg for ARL 15895AR and 32 mg/kg for ARL 15896AR. The differences in neurobehavioral effects between these enantiomers may be related to brain levels since a pharmacokinetic study reported ARL 15895AR to have a greater brain:plasma ratio than ARL 15896AR at 5 minutes after an intravenous dose.

175 ENANTIOMERIC DIFFERENCES IN RATS IN THE TOXICOKINETICS (TK) OF THE NEUROPROTECTIVE DRUGS (S) AND (R) α-PHENYL-2-PYRIDINEETHANAMINE.2HCI: ARL 15896AR (S) AND ARL 15895AR (R)

D J McCarthy, M S Eisman, S B Rawleigh, and S H Curry. *Preclinical Research Support, Astra Arcus USA Rochester, NY*

ARL 15896AR (S) and ARL 15895AR (R), have been evaluated in *in vitro* and *in vivo* models believed to be predictive of efficacy in stroke, neuroprotection, and epilepsy. ARL 15896AR (S), has a better safety margin and more predictable pharmacokinetics in rats than ARL 15895AR (R). We determined plasma concentrations of these enantiomers in male and female Sprague-Dawley rats during a rising dose TK study in which rats received a single daily i.v. dose for six weeks. Rats received 3 mg/kg of the free base of ARL 15896AR for four days starting on day 0, followed by progressively increasing doses of 6, 9, 12, 15, 20, 25, 30, and 36 mg/kg. Rats received 3 mg/kg of the free base of ARL 15895AR for four days starting on day 0, followed by progressively increasing doses of 6, 9, and 12 mg/kg. At each ascending dose change, blood was taken from a single rat at 0.25, 0.5, 4, and 24 hours after dosing. The plasma concentrations of ARL 15896AR but not ARL 15895AR, were proportional to the dose administered in both males and females. The estimated elimination half-life of ARL 15896AR did not change after repeated dosing and could not be estimated for ARL 15895AR. No accumulation of the compounds occurred in rats after six weeks repeated dosing. No differences were observed in the TK of the compounds between males and females. There was no interconversion of ARL 15896AR to ARL 15895AR *in vivo*.

176 EFFECT OF HEMORRHAGE AND FLUID REPLACEMENT ON BLOOD ETHANOL (ETOH) ELIMINATION

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ETOH is absorbed, rapidly distributed and eliminated according to zero order kinetics. The effects of blood loss and fluid replacement on blood ETOH concentration are of medical and forensic importance. Studies in animals were conducted on Sprague Dawley rats, 300–350 g. Rats were anesthetized and catheterization of femoral vein was accomplished 48 hr prior to i.v. administration of ETOH (0.57 g/kg). Blood (1.0 ml/100 g bw) was withdrawn at 35 min. Groups of animals were administered ETOH with no hemorrhage, hemorrhage and hemorrhage with donor blood or fluid replacement (lactated Ringer's solution). Blood samples were collected at 0, 30, 35, 40, 45, 55, 65 and 90 min for determination of ETOH and hematocrit.

The blood concentration of ETOH decreased in all treated groups of animals at a rate which was not statistically different from that of the control group. These results are attributable to rapid transfer of ETOH from the extravascular spaces to circulating blood.

177 CHRONIC TOXICITY AND TOXICOKINETIC (TK) STUDIES OF PC1358 IN RATS AND DOGS

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PC1358 is a peptide-copper complex under development as a topical treatment for hair loss (androgenetic alopecia, alopecia areata, secondary alopecia). Studies were conducted to assess the toxicity and TK profile. PC1358 was applied twice daily for 26 weeks in equally divided doses within a clipped dorsal area that was 60 cm^2 for dogs and 6 cm^2 for rats. Dogs (4/sex/group) received 0 (vehicle), 4, 10, or 20 mg/kg/day at a volume of 0.4 mL/kg/day; rats (15/sex/group) received 0 (vehicle), 10, 25, or 50 mg/kg/day at a volume of 1.0 mL/kg/day. Mean dermal doses were 0.2 to 0.9, 0.5 to 2.2, and 1.0 to 4.5 mg/cm^2 for rats and 0.3 to 0.7, 0.9 to 1.8, and 1.9 to 3.4 mg/cm^2 for dogs. Animals received food and water ad libitum, were observed twice daily for mortality and moribundity, and had body weights and food consumption recorded weekly. Dermal irritation was scored daily during the first week of treatment and weekly thereafter. Clinical pathology tests and TK analyses were performed mid-study and prior to scheduled sacrifice. There were no test material-related findings indicative of systemic toxicity. There was a dose-related increase in dermal irritation corresponding to histological findings of epidermal hyperplasia at the highest dose level in rats and at all dose levels in dogs (except vehicle). Plasma levels of copper were within background. Based on the results, the NOEL for systemic toxicity was 20 mg/kg/day in dogs and 50 mg/kg/day in rats.

178 METHYL *tert*-BUTYL ETHER (MTBE) vs ETHYL *tert*-BUTYL ETHER (ETBE): A COMPARISON OF BLOOD AND TISSUE PARTITION COEFFICIENTS IN MALE AND FEMALE F-344 RATS

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MTBE and ETBE are structurally similar compounds used as gasoline additives to decrease CO emissions. MTBE causes renal tumors in chronically exposed male rats, whereas ETBE has not been tested. In development of physiologically based pharmacokinetic models to assess the kinetic behavior of MTBE and ETBE in male and female rats, one of the primary data requirements is partition coefficients (PC) for the chemical in each tissue group displaying distinct kinetic behavior. In this study, PC were measured in blood, liver, kidney, fat, and muscle of male and female F-344 rats using an *in vitro* vial equilibration method. Liver and kidney samples were preheated (60°C for 1 hr) prior to measuring the PC to ensure that metabolism did not occur during the incubation period. Heat treatment did not affect the uptake of MTBE or ETBE into the male or female rat liver but did cause a measurable decrease in the PC of MTBE and ETBE in the male but not female rat kidney. The PC of MTBE was higher in blood and liver and lower in fat of both sexes compared with ETBE. Both MTBE and ETBE were ~5–10 times more soluble in male rat kidney when compared with female rat kidney. Factors contributing to the differences in the measured PC in male and female rat kidney are unclear. However, the greater solubility of ETBE in fat and its lower solubility in blood compared with MTBE suggests that ETBE would be cleared from blood of exposed rats at a slower rate than MTBE. (This work was sponsored in part by the Oxygenated Fuels Association.)

179 HEPATIC BLOOD FLOW LIMITATION OF THE BIOACTIVATION OF HAZARDOUS CHEMICAL AIR POLLUTANTS

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The U.S. EPA has identified 189 substances as hazardous air pollutants (HAPs) in the Clean Air Act Amendments of 1990, including many organic chemicals that are bioactivated by cytochrome P450 2E1. The P450 2E1-mediated bioactivation of chemicals typically occurs with high rates and affinities. Dynamic interindividual variations in P450 2E1 are expected since this enzyme is readily induced by exposure to alcohols, ketones, and heterocycles through diet and lifestyle. The default assumptions used by the U.S. EPA in risk assessments include uncertainty factors for interindividual differences in bioactivation. This study utilized physiologically based pharmacokinetic models and literature data to determine the effect of enzyme induction

on the bioactivation of 18 HAPs by rats and humans. The liver concentration of metabolite was simulated after inhalation exposure to 10 ppm HAP. Tenfold increases in Vmax had little or no effect on the bioactivation of 1,1,2,2-tetrachloroethane, 1,1,2-tri-chloroethane, 1,2-dichloroethane, 1,4-dioxane, acrylonitrile, chloroform, styrene, trichloroethylene, vinyl chloride, or vinylidene chloride due to hepatic blood flow limiting delivery of the HAP to the liver. HAPs that did not exhibit blood flow limitation included butadiene, benzene, carbon tetrachloride, and tetrachloroethylene. The hepatic blood flow limitation of HAP bioactivation indicates that interindividual differences in P450 2E1 due to induction will not affect toxic metabolite formation. The limiting effect of hepatic blood flow on bioactivation should be considered in risk assessments of HAPs.

180 AN ANALYSIS OF INTER-INDIVIDUAL VARIABILITY IN UPTAKE AND ELIMINATION OF CHROMIUM FROM HUMAN VOLUNTEERS

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Human volunteers were exposed to varied amounts of selected trivalent [Cr(III)] and hexavalent [Cr(VI)] chromium compounds in water at concentrations up to 10 mg Cr/L, and blood and urine samples were collected for analysis of chromium concentrations for up to 14 days following exposure. Classic pharmacokinetic data analysis techniques were used to examine the influence of a variety of parameters on chromium absorption rate and elimination half-life, such as chromium concentration, volume administered, multiple dose administration, and possible interactions of diet and the timing of chromium ingestion. The results indicated that there were significant differences in the absorption and elimination of hexavalent and trivalent chromium among the volunteers. These differences seemed to depend primarily on dietary patterns and possibly the contents of the stomach at time of chromium ingestion. The percent absorption and the time course of elimination appeared to have been affected as to whether ingestion occurred when fasted or following a meal. These differences were generally more substantial than the interindividual variability in the elimination half-lives among the volunteers. Since the data were collected through at least five half-lives and the limits of detection of the analyses were relatively low, these results may represent the best estimates of the plausible range for the human elimination half-life and oral absorption rate of Cr(III) versus Cr(VI).

181 P-GLYCOPROTEIN DISTRIBUTION AND INDUCIBILITY IN THE CATFISH INTESTINE: ROLE IN BENZO(A)PYRENE (BaP) TRANSPORT

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P-glycoprotein (pgp) is a 170 kDa plasma membrane bound efflux pump known to transport a variety of bulky xenobiotics. Pgp appears to facilitate xenobiotic excretion into the bile and urine, however, it's role in the intestine has not been clearly defined. This study examined in the catfish intestine: 1) the regional distribution of pgp; 2) the responsiveness of intestinal pgp to the prototypic pgp inducer vincristine (VIN) (20 μg/kg diet) 3) the responsiveness of pgp to the P450 1A inducer β-naphthoflavone (BNF) (10 mg/kg diet) and 4) the role of pgp in the transport of dietary [^{3}H] benzo(a)pyrene (BaP) (2μM). Regional distribution and inducibility was examined with immunohistochemistry (Mdr-1/Mdr-3; C-219 clone) and image analysis. The influence of pgp on [^{3}H] BaP bioavailability was evaluated using an *in situ* isolated perfused intestinal segment of BNF induced catfish with and without the pgp inhibitor verapamil (20μM). For all dietary treatments the distal intestine exhibited significantly higher levels of pgp than the proximal intestine. Dietary pretreatment with VIN and BNF for 14 days resulted in 6.4(VIN)/7.8 (BNF) fold and 2.4 (VIN)/2.8 (BNF) fold increases in luminal pgp for the proximal and distal intestine, respectively. Treatment of the *in situ* intestinal preparation with verapamil under the conditions described did not alter the systemic availability of BaP from the intestine. This study demonstrated the inducibility of intestinal pgp with dietary VIN and BNF, regional differences in intestinal pgp distribution and inducibility and a limited effect of pgp transport upon the systemic bioavailability of BaP. (P42 ES 07375)

182 COMPARISON OF THE METABOLISM AND TISSUE DISTRIBUTION OF 14C-LABELED SPINOSYN A AND 14C-LABELED SPINOSYN D IN FISCHER 344 RATS

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Spinosad is a natural fermentation product produced by Saccharopolyspora spinosa. This insect control agent is a mixture of Spinosyns A and D with Spinosyn A representing the predominant moiety. The data on the absorption, distribution metabolism and excretion of Spinosyn A were compared with the data from Spinosyn D. There were no major differences between the bioavailability, routes or rates of excretion, or metabolism of 14C-Spinosyn A and 14C-Spinosyn D in Fischer 344 rats following oral administration as a suspension at 100 mg 14C-Spinosyn A or D/kg body weight. The major elimination route was fecal excretion for both Spinosyns. Approximately 80% and ~66% of the administered dose was absorbed for Spinosyn A and D, respectively, with ~20% (Spinosyn A) and ~34% (Spinosyn D) of the dose eliminated unabsorbed in the feces. By 48 hr post-dosing, pimt60% (Spinosyn A) and pimt80% (Spinosyn D) of the administered dose was recovered in the urine and feces. Based on the terminal half-lives for urinary and fecal excretion the elimination half-life for Spinosyn A ranged from 25-42 hr and the half-life for Spinosyn D ranged from 29–33 hr. The tissues and carcass contained very low levels of radioactivity at 168 hr post-dosing, less than 0.1% of the administered dose per gram of tissue. The primary fecal, urinary and the biliary metabolites associated with Spinosyns A and D were identified as the glutathione conjugates of the parent, N- demethylated and Odemethylated Spinosyns A and D. In summary, the absorption, distribution, metabolism and elimination of Spinosyns A and D in Fischer 344 rats were similar.

183 COMPARATIVE PHARMACOKINETICS OF INHALED AND INTRAPERITONEAL PYRIDINE AND ACETONE AND ASSOCIATED CYPIA INDUCTION IN THE RAT

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The pharmacokinetics of pyridine or Acetone was determined following exposure to (i) single or three consecutive ip doses of either pyridine (100 mg/kg) or acetone (400 mg/kg) or (ii) pyridine vapor (200 ppm) or acetone vapor (1000 ppm) administered 8 h/day for 3 consecutive days. Plasma samples were obtained at 0,0.5,1,2,4,8,16, and 24 h following termination of exposure. Maximal plasma concentration of pyridine was 0.10 mg/ml at 1 h and 0.23 mg/ml at 0 h following i.p. and inhalation administration, respectively. Plasma half-life for pyridine was 17 h and 12 h following one and three consecutive i.p. doses, respectively and 7.5 h following 3 daily inhalation doses. Maximal plasma levels of acetone were 0.29 mg/ml (1 h) and 0.11 mg/ml (0 h) following ip and inhalation exposure, respectively; corresponding plasma half-life was 4.1 h and 4.7 h. The data suggest no major differences between the studied routes of exposure in the pharmacokinetics of the chemicals. Similarly, no major differences were observed in the induction of CYP1A1 and CYP1A2 (as measured by catalytic activities, protein levels and mRNA levels) by the different routes of exposure. Supported by ES06414.

184 DISPOSITION OF DIMETHYLARSINIC ACID IN THE MOUSE AFTER INTRAVENOUS ADMINISTRATION

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Dimethylarsinic acid (DMA) is the predominant mammalian metabolite detected in urine after exposure to arsenate. Previous studies have suggested there is a dose-dependent disposition of arsenate in the mouse. The primary aim of this study was to evaluate if the dose-dependency is due to saturation of urinary excretion of DMA. Female B6C3F1 mice (3/cage, 3 cages/dose) were administered 1.11 or 111 mg/kg of [^{14}C]-DMA iv. Excreta was collected for 24 hr and the mice were sacrificed. Tissues were excised and analyzed for ^{14}C. Greater than 75% of the dose at both levels was excreted in the urine by 24 hr. Within 2 hr, greater than 50% of the urinary excreted dose had been detected. No effects on the total % dose excreted were detected. Fecal excretion was 2–4% of the dose, and was significantly greater in the low dose group. Tissue ^{14}C was low (<1% of the dose) and based on % dose was highest in liver followed by kidney and lung. Concentration of ^{14}C (% dose/gm tissue) was higher in the tissues than in blood, indicating that DMA can partition into these tissues. No dose effects on the distribution of DMA were observed. The lack of a dose effect on urinary excretion of DMA,

suggests the dose-dependent effect of arsenate disposition may be due to effects on metabolism of arsenate. (This abstract does not reflect EPA policy.)

185 TOXICOKINETICS OF PEG_{12000}-INTERFERON (IFN) ALFA-2B GIVEN BY SUBCUTANEOUS (SC) INJECTION TO CYNOMOLGUS MONKEYS FOR ONE MONTH

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Intron® A (IFN alfa-2b) is marketed worldwide for the treatment of hepatitis C. Treatment consists of SC injections three times per week due to its short half-life (approx. 4 hours). A polyethylene glycol-12,000 (PEG_{12000}-IFN) conjugate has been developed with the goal of producing a long-acting product which would require only once weekly injections. Cynomolgus monkeys (N = 3/sex) were given PEG_{12000}-IFN at SC doses of 1414, 4239, or 14126 μg IFN protein per square meter of body surface area (μg/m²), or Intron® A at 3105 μg/m², every other day for one month. Multiple blood samples were collected on Day 1, on Day 5, and during Week 4 for determination of serum IFN-equivalent concentrations using both a bioassay (Cytopathic Effect Assay, or CPE) and an enzyme immunoassay (EIA). Serum samples were also collected 24 hours after the last dose as well as after a four-week postdose period, and were analyzed for serum neutralizing factors (SNF) to IFN by both CPE and EIA. Serum IFN-equivalent concentrations (both C_{max} and AUC) following PEG_{12000}-IFN dosing were dose-related, gender-independent, and reached a C_{max} within 4–8 hours. Day 1 serum concentrations were quantifiable to 48 hours postdose at all dose levels of PEG_{12000}-IFN; in contrast, serum concentrations following dosing of Intron® A were near baseline after 24 hours. Moreover, PEG_{12000}-IFN provided 3–5 times the systemic exposure of Intron® A based on AUC values. Day 5 serum PEG_{12000}-IFN concentrations showed accumulation in the range of 10–60%. Serum SNF analysis showed that monkeys in all PEG_{12000}-IFN or Intron® A dose groups developed neutralizing activity to IFN by postdose Week 4 and was likely already present by Week 4 of dosing. PEG_{12000}-IFN has the toxicokinetic properties to be a long-acting IFN product in the treatment of hepatitis C.

186 MECHANISMS OF XENOBIOTIC INCORPORATION INTO HAIR: PHENCYCLIDINE

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The incorporation of phencyclidine (PCP) into the hair of Long-Evans rats was investigated to evaluate its utility as a model xenobiotic for mechanistic studies. PCP was given by ip injection daily for 5 days and pigmented and nonpigmented hair were collected 14 days later and analyzed for PCP by GC-ion trap mass spectrometry. PCP concentrations in pigmented hair were 2.86 ± 0.55, 10.30 ± 1.15, 14.33 ± 1.43, and 29.20 ± 6.02 ng/mg of hair after doses of 1, 4, 12, and 24 mg/kg, respectively; PCP concentrations in nonpigmented hair were 0.30 ± 0.07, 0.47 ± 0.04 and 1.05 ± 0.39 after 4, 12, and 24 mg/kg, respectively. PCP was not detectable in nonpigmented hair after a dose of 1 mg/kg daily for 5 days.

Pigmented hair from previously dosed animals was pooled and washed daily for ten days with either deionized water or 1% SDS detergent. Hair aliquots were obtained daily from these protocols and analyzed in triplicate for PCP. PCP concentrations for non-washed pigmented hair were 14.91 ± 0.98 ng/mg of hair. No significant differences were seen when daily aliquots of washed hair were analyzed and compared to the non-washed hair.

This study demonstrates that PCP incorporates into pigmented and nonpigmented hair in a dose proportional manner with a 30 fold preference for pigmented hair. Once incorporated into hair, PCP is resistant to washing with either water or detergent. Supported by NIDA grant #DA 07820.

187 COMPARATIVE PHARMACOKINETICS OF TRICLOPYR (TR) GIVEN AS THE ACID, BUTOXYETHYL ESTER (TR-BEE) OR TRIETHYLAMINE (TR-TEA) SALT TO FISCHER 344 RATS

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The plasma kinetics, metabolism and tissue distribution of 14C-TR were similar following equimolar oral doses of the acid (1, 3 or 60 mg/kg) or TR-BEE (1.4, 4 or 83 mg/kg) in corn oil. Likewise, the kinetics of equimolar oral doses of 14C-TR (3 mg/kg) and TEA (1.5 mg/kg) in H_2O did not differ when TR and TEA were given alone or in combination. In all cases, 14C-TR was rapidly absorbed with peak plasma 14C levels reached by ~3 hr and ~5–6 hr post-dosing for low and high dose, respectively, of TR and TR-BEE in corn oil, and by ~0.5 hr post-dosing with TR and TR•TEA in water. Plasma AUCs and clearance, elimination $t_{1/2}$ and apparent volume of distribution were similar for the low doses of the acid and TR-BEE. Distribution of 14C in the tissues at peak plasma 14C concentration (evaluated at the high dose) were similar for the acid and TR-BEE. All forms of 14C-TR were rapidly eliminated principally unchanged as TR in the urine (>74% of the 14C dose within 24 hr). However, the urinary elimination of 14C-TR was apparently saturated during the first 12 hr after the high dose of acid and TR-BEE. The feces accounted for <5% and, after 24-72 hr, the tissue and carcass <3% of the dose. Overall these data indicate that the fate of the TR moiety in TR-BEE and TR-TEA was unchanged relative to that of the acid. These results are consistent with the similar results obtained in a variety of toxicity studies conducted with TR compared to those of TR-BEE and TR-TEA.

188 A 3-WEEK INTERACTION STUDY OF rhuMAb HER2 WITH DOXORUBICIN, CYCLOPHOSPHAMIDE AND PACLITAXEL IN RHESUS MONKEYS

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rhuMAb HER2 is a humanized monoclonal antibody currently in Phase III clinical trials in breast cancer patients with $p185^{HER2}$ over-expression. A disposition study was conducted in rhesus monkeys with rhuMAb HER2 and 3 chemotherapeutic agents to detect in vivo interactions. Twenty-one female rhesus monkeys were randomized into 7 groups and administered a single dose of rhuMAb HER2 alone (1.5 mg/kg), or each of the following chemotherapeutic agents in the presence or absence of 1.5 mg/kg rhuMAb HER2: doxorubicin (1.5 mg/kg), paclitaxel (4 mg/kg) or the combination of doxorubicin (1.5 mg/kg) and cyclophosphamide (15 mg/kg). The dose for each test material approximates the human dose on a body-weight basis; paclitaxel was administered as a 1-hour infusion and the remaining test materials were administered by intravenous bolus injection. Clinical signs, body weight, body temperature, heart rate, respiration rate, ECG, hematology, serum chemistry, urinalysis and antibody formation to rhuMAb HER2 were monitored. Blood samples were collected for drug concentration analysis at the following intervals: pretreatment and at 5, 15, 30, 45 minutes, 1, 2, 3, 4, 6, 8, 12, 16, 24, 48, 72 hours and 4, 5, 6, 7, 9, 11, 14, 17 and 21 days postdose. Administration of rhuMAb HER2 alone or in combination with the chemotherapeutic agents was well tolerated and produced no evidence of systemic toxicity. There was no evidence of differential disposition of rhuMAb HER2 in the presence of doxorubicin, paclitaxel or doxorubicin + cyclophosphamide; conversely, administration of rhuMAb HER2 did not affect the disposition of the chemotherapeutic agents.

189 EFFECT OF PROBENECID ON VALPROIC ACID STEADY-STATE DISPOSITION IN RATS

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Previous investigations have demonstrated that valproic acid (VPA) and probenecid (PRB) bind moderately to plasma proteins and that PRB inhibits VPA glucuronidation. The purpose of the present study was to examine the effects of PRB on VPA protein binding and disposition. Male Sprague-Dawley rats (290–390 g) were anesthetized with urethane (1 g/kg i.p.); the jugular/femoral veins and bile duct were cannulated. VPA (5–360 mg/kg bolus + 0.19–4.85 mg/kg/min infusion) and PRB (n = 6; 50 mg/kg bolus + 13 mg/kg/hr infusion) or saline (n = 6; 2.0 ml/kg bolus + 0.33 ml/kg/hr infusion) were co-administered via the femoral cannula for 60 min. Blood samples (250 μl) were collected and VPA concentrations were quantitated by gas chromatography. *Ex vivo* binding was assessed by ultrafiltration, and binding parameters were determined by nonlinear least squares regression. PRB decreased the association constant for VPA binding ~2-fold (from 3.26 to 1.66 ml/mg), consistent with competitive displacement from protein binding sites; binding capacity was not altered substantially by PRB. Steady-state VPA clearance decreased with increasing total VPA levels both in the absence (from 2.65 to 1.26 ml/min at VPA concentrations of 132 to 1297 μg/ml and presence (from 1.92 to 0.96 ml/min at VPA concentrations of 36.5 to 851

μg/ml) of PRB. The difference in VPA clearance between saline- and PRB-treated rats at similar VPA concentrations was consistent with inhibition of VPA glucuronidation, as observed in previous *in vitro* experiments. The effect of PRB on the hepatobiliary disposition of VPA is currently being investigated. Funding: NIH GM41935; NIEHS T32 ES07126.

190 THE EFFECT OF WATER RESTRICTION ON BSP DISPOSITION IN THE RAT

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The metabolism of various model drugs (antipyrine, acetaminophen) is impaired in dehydrated rats. In the present study we investigated the disposition of sulfobromophthalein (BSP) in water-restricted (WR) rats. 3 groups of rats (n = 6) were maintained in metabolic cages. The control rats (C) had water and food ad lib; the WR rats received 20% of the water consumed by C rats, for 10 days. The rehydrated (RE) rats had the same water-ration as the WR rats, however, on the 11th day they received water ad lib. BSP was injected IV (100 mg/kg). Blood samples were taken up to 60 min. BSP was determined in plasma, liver and intestines, spectrophotometrically. The $t_{1/2}$ of BSP was significantly longer in WR rats as compared to C and RE rats (22.55 ± 2.55 vs 9.01 ± 0.96 and 11.73 ± 1.76 min respectively). The clearance of BSP in WR and RE rats was lower as compared to C rats (4.307 ± 0.316 and 5.251 ± 0.481 vs 8.051 ± 0.633ml/min/kg respectively). The AUC of BSP in WR and RE rats was bigger than that of C rats (20.1 ± 1.8 and 16.0 ± 2.0 vs 10.7 ± 0.9 mg*min/ml respectively). The retention of BSP in the liver of WR rats was higher than that in the C and RE rats (38.7 ± 8.2 and 25.4 ± 5.2 vs 8.6 ± 2.0 ug/g liver) while the intestinal levels of BSP were lower in WR rats as compared to C and WR rats (1.35 ± 0.14 vs 1.96 ± 0.12 and 2.07 ± 0.14 mg/100g BW). These results indicate that the elimination of BSP in WR rats is impaired in comparison to C rats. One day of rehydration only partially improved the elimination of BSP as compared to WR rats. Because the clearance of BSP is hepatic blood flow dependent, it may be concluded that water restriction impairs blood flow to the liver. Reduced hepatic blood flow and slower disposition may increase the toxicity of several drugs whose clearance is flow dependent.

191 METABOLISM OF COCAINE AND HEROIN IS REGULATED BY THE SAME HUMAN LIVER CARBOXYLESTERASES

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Concomitant intravenous use of cocaine and heroin ("speedballing") is prevalent among drug abusing populations. Heroin is rapidly metabolized by sequential deacetylation of two separate ester bonds to yield 6-monoacetylmorphine (6-MAM) and morphine. Hydrolysis of heroin to 6-MAM is catalyzed by pseudocholinesterase (PChE). The mechanism for hydrolysis of 6-MAM to morphine has yet to be established. PChE and two human liver carboxylesterases (hCE-1 and hCE-2) catalyze the rapid hydrolysis of ester linkages on cocaine. This investigation examined the potential contribution of hCE-1, hCE-2 and PChE to heroin metabolism and explored interactions between heroin and cocaine metabolism. Enzymatic hydrolysis of heroin to 6-MAM and 6-MAM to morphine was determined by reversed-phase HPLC. All three enzymes rapidly catalyzed hydrolysis of heroin to 6-MAM (hCE-1 kcat = 439 min^{-1}, hCE-2 kcat = 2186 min^{-1}, PChE kcat = 13 min^{-1}). From steady-state kinetic analysis, the catalytic efficiency for hCE-2 catalyzed formation of 6-MAM (314 min^{-1} mM^{-1}) was much greater than that for either hCE-1 or PChE (69 and 4 min^{-1} mM^{-1}, respectively). Similarly, the catalytic efficiency for hydrolysis of 6-MAM to morphine by hCE-2 (22 min^{-1} mM^{-1}) was greater than that for hCE-1 (0.024 min^{-1} mM^{-1}). Cocaine competitively inhibited hCE-1 and hCE-2 catalyzed hydrolysis to 6-MAM (K_i = 930 and 680 μM, respectively) and hCE-2 hydrolysis to morphine (K_i = 230 μM). These data suggest that the duration and severity of the combined pharmacological actions of cocaine and heroin in man are regulated by common metabolic pathways mediated by these hepatic carboxylesterases and PChE. (Supported by DA 06836 and DA 08215).

192 RETENTION OF CONTININE AND ACTIVATION OF PAF-HYDROLASE, POSSIBLE RISK FACTORS FOR SMOKING-INDUCED ARTERIAL THROMBOSIS IN THE RAT

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Cotinine is an oxidized metabolite of nicotine and has a long biological half-life (19–24 h) in man. Its plasma concentration is used as an index of tobacco smoke exposure. The turnover rate of platelet activating factor (PAF) is regulated by PAF-hydrolase(**PAF-H**), which increases in the plasma of hypertensive rats. In order to evaluate the role of cotinine as a risk factor for arterial thrombosis, we have studied the retention of cotinine formed after administration of nicotine and activation of PAF-H of rat platelets and spleen microsomes. [^{14}C]-nicotine (1mg/kg, 5μCi/kg) was administered into femoral artery and retention times of nicotine and cotinine were determined. The plasma level of nicotine reached peak levels in 5–10 minutes and then declined. Cotinine levels remained constant during 5–60 minutes. PAF-H activity of rat platelets and spleen was assayed by a radiometric method using ^{3}H-PAF and recovering lyso-^{3}H-PAF. Cotinine or nicotine (10μM) activated PAF-H *in vitro* by 70% and 55% respectively. These observations indicate that accumulation and retention of cotinine in plasma is a risk factor for the occurrence of arterial thrombosis with high frequency in smokers. (Supported by the Smokeless Tobacco Research Council and the Study Center for Anesthesia Toxicology).

193 TOXICOKINETICS OF METHYL CHLORIDE IN RELATION TO POLYMORPHIC DISTRIBUTION OF GLUTATHIONE-*S*-TRANSFERASE T1 IN VOLUNTEERS

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Interindividual variation in the conjugation of methyl chloride with glutathione has been observed in humans. This variation probably depends on a polymorphic variation in glutathione-*S*-transferase T1 (GSTT1) activity. Polymorphic distribution of GSTT1 has been demonstrated *in vitro* in human blood with methyl chloride as substrate. Homozygosity for a deleted GSTT1 gene has also been determined using a PCR-method. The aim of this study was to investigate if individuals who lack GSTT1 also have lower metabolism *in vivo*.

Male and female volunteers, 8 with high (+ +), 8 with intermediate (+ −), and 8 with no (+ −) glutathione-*S*-transferase activity as determined by phenotyping and genotyping were exposed to methyl chloride vapors (10 ppm, 2h) in an exposure chamber. Uptake and disposition was studied by measuring methyl chloride in inhaled air, exhaled air, and blood.

A preliminary analysis of the experiments suggests a marked difference between (+ +) and (− −) individuals. Markedly lower uptake (1% versus 20%), slightly higher blood concentration, and higher post-exposure exhalation of methyl chloride indicate slower metabolism of methyl chloride in the (− −) subjects compared to the (+ +) subjects.

Genetic variation in GSTT1 in erythrocytes was confirmed to be important for the *in vivo* metabolism of methyl chloride.

194 ARSENITE-INDUCIBLE PROTEINS IN HUMAN LYMPHOBLASTOID CELL LINES MAY CORRELATE WITH CLASTOGENIC EFFECTS

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We have shown inter-individual variation in arsenite (As^{III}) induced sister chromatid exchange (SCE) among human lymphoblastoid cell lines. Using 2D PAGE we found that heat (43°C, 15 min) induced typical heat shock proteins (HSP) in these cell lines. As^{III} (5 μM) also induced some of the HSP. The 2 treatments both increased SCE frequency and together were additive in increasing SCEs. As judged from the 2D gels, As^{III} and heat both produced increases in 5 proteins (21, 52, 58, 79 and 86 kD). However, As^{III} induced at least 1 unique protein (98 kD), and did not induce 1 which was induced by heat (66 kD). Between 2 cell lines showing differing sensitivity to the clastogenic effects of As^{III}, the most sensitive line was also most responsive to induction of those proteins induced by either As^{III} or heat. The results suggest that individual sensitivity to the clastogenic effects of As^{III} is genetically determined. Further, since the proteins induced by heat and As^{III} are not all identical, an arsenic-specific response is suggested. Supported in part by NIH Training Grant #ESO7157.

195 THE DISPOSITION AND METABOLISM OF 2-METHYL-IMIDAZOLE IN RATS

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2-Methylimidazole (2-MI), is widely used as a chemical intermediate, is present in cigarette smoke, and may form in food as a result of ammoniation of simple sugars. 2 - MI has been shown to be neurotoxic in several animal species and to affect serum levels of T3, T4, and TSH in the rat, apparently leading to hyperplasia of thyroid follicular cells. In order to better characterize 2-MI-induced toxicity, the disposition and metabolism of [2- ^{14}C] 2 - MI have been investigated following po administration of 5, 50, and 150 mg/kg to male F344 rats. Excretion data indicated that absorption of 2-MI was both rapid and proportional to dose in the range studied. About 90% of the total dose was eliminated in urine within 24 hr of gavage. Most of the remaining ^{14}C was excreted in feces and as expired $^{14}CO_2$. Excretion data was similar following iv administration of 5 mg/kg, indicating no effect of dose route on 2-MI disposition. Little or no enterohepatic circulation of dose occurred, since biliary excretion of 2 -MI-derived ^{14}C was negligible. About 70% of the ^{14}C excreted in urine consisted of parent compound. HPLC chromatograms for all treatment groups were similar, indicating that metabolism of 2-MI in rats was not affected by dose or route of administration. Increased UDP-glucuronyl transferase activity was found in livers of rats receiving 2-MI at 10,000 ppm in dosed-feed for 29 days. Therefore, 2-MI-mediated thyroid hyperplasia may involve enhanced metabolism of thyroid hormones by these enzymes.

196 BIOAVAILABILITY OF DUAL LABELED CHOLESTAGEL™ IN DOGS AND RATS AFTER ACUTE AND SUBCHRONIC DRUG ADMINISTRATION

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CholestaGel™, a cross linked terpolymer of N-decyl-N-allylamine, N-(6-trimethylammoniumhexyl)-N-allylamine and allylamine is a non-metabolizable highly efficacious bile acid sequestrant. The activity of a bile acid sequestrant is confined to the lumen of the GI tract. Villous tip atrophy has been observed in the intestines of animals subchronically treated with other bile acid sequestrants raising questions about potential drug absorption after subchronic dosing. To demonstrate that CholestaGel is non absorbed after either acute or subchronic administration, the polymer was labeled with ^{3}H in the allylamine backbone and ^{14}C in the trimethylammonium side chain. Beagle dogs and Sprague Dawley rats were administered radiolabeled polymer (4.7 μCi ^{14}C/kg and 10.7 μCi ^{3}H/kg in dogs; 46.8 μCi ^{14}C/kg and 95.2 μCi ^{3}H/kg in rats) acutely or following 28 consecutive days of unlabeled polymer treatment (1200 or 3000 mg/kg/day in rats; 1200 mg/kg/day in dogs). Blood, urine and fecal samples were collected at timepoints up to 72 hours and tissues were harvested at the time of sacrifice. From these data, the percent dose excreted in urine and feces, percent dose remaining in whole blood and harvested tissue, and total recovered radioactivity were calculated. No significant urinary excretion of radioactivity was observed in any of the treatment groups in either rats or dogs. The mean fraction of dose observed in the whole blood, liver, spleen, lymph nodes and muscle was 0.000%. Fecal excretion data and total recovery data indicate that [^{3}H, ^{14}C]-Cholesta-Gel-derived radioactivity was entirely excreted in the feces. These data demonstrate non-absorbability of [^{3}H, ^{14}C]-CholestaGel.

197 SEX-DEPENDENT DIFFERENCES IN THE IV PLASMA KINETICS AND ORAL DISPOSITION OF 2,4-D IN SPRAGUE-DAWLEY RATS

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2,4-Dichiorophenoxyacetic acid (2,4-D), a widely used broadleaf herbicide, is currently under investigation by the National Toxicology Program in a study of peroxisome proliferators. As part of this study male and female Sprague-Dawley rats, were dosed with ^{14}C-2,4-D orally at 5 and 200 mg/kg (20 μCi) and tissue distributions were determined at 2, 8, 24, and 72 hours post dosing. Blood, liver, kidney, muscle, skin, fat, brain, testes and ovaries were examined. At early timepoints female tissues contained markedly and consistently higher amounts of radiaoctivity than did male tissues (up to 9X). By 72 hours, tissue levels were equivalent and males and females had excreted equal amounts of radioactivity into the urine. Differences observed at earlier time points were hypothesized to be due to more rapid clearance of 2,4-D by males. To test this, plasma elimination curves were generated in male and female rats following iv administration of ^{14}C-2,4-D (5 μCi) through a jugular vein cannula. Kinetic analysis revealed significant differences in AUC, Cl and MRT consistent with a greater ability to clear 2,4-D in males relative to females. This suggests that at equivalent doses, female rats are exposed to higher concentrations of 2,4-D for a longer time than males and may be more susceptible to any toxic effects than males. If this sex difference extends to humans, it would be of great importance for human risk assessment.

198 PHARMACOKINETICS AND DISPOSITION OF [2-^{14}C]NMP

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N-Methyl-2-pyrrolidinone (NMP) is a widely used organic solvent. The object of this study was to investigate the pharmacokinetics and disposition of NMP after IV (50 mg/kg), and oral (5, 50 mg/kg) exposures to male and female rats. Plasma levels were analyzed through 120 hours after exposure; the data suggested rapid distribution and elimination phase. Dispositional half life for intact NMP ranged between 1 and 2.5 hours. The absolute bioavailability of NMP was measured. The major route of excretion was via the urine which accounted for approximately 80% with more than 50% of the dose within the first 12 hours post exposure. The radioactivity in tissues and organs at 120 hours post exposure was negligible with the highest amounts in the liver, lung and kidney. Radiomonitored HPLC analysis of urine revealed one major and two minor metabolites; their identity is currently being investigated. We are continuing these studies examining both dermal and inhalation exposures.

199 PREDICTING THE *IN VIVO* METABOLIC CLEARANCE OF DRUGS FROM *IN VITRO* DRUG METABOLISM DATA

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Advances in *in vitro* technology and analytical chemistry provide opportunities to study drug metabolism in greater detail. Such *in vitro* data can be used to predict *in vivo* behavior or, importantly, hepatic clearance (CL_H). We tested the hypothesis that *in vitro* data can be used to predict *in vivo* hepatic clearance. We used two *in vitro* tools: isolated perfused rat liver (IPRL) in the single pass mode and precision-cut liver slices. All experiments were performed using male Fisher rats (270–310g). The experimental data were then compared to literature values of clearance obtained from pharmacokinetic studies. Wherever possible, the data were scaled to humans. The CL_H and intrinsic clearance (CL_{int}) were calculated. The extraction ratio (ER) of cocaine (COC), benzoic acid (BA) and tolbutamide (TOL) were measured using the IPRL. The ER of COC[1] was 0.88 (CL_H = 22 ml/min; CL_{int} = 183 ml/min). The *in vivo* Cl_{int}[2] was 144 ml/min. The ER of BA was 0.35 (CL_{int} = 3.7 ml/min). The corresponding ER in humans was 0.32 (70 kg human). The ER of TOL was estimated as 0.21 (CL_{int} = 0.55 ml/min.g). The *in vivo*[3] ER value is 0.020 (CL_{int} = 0.1 ml/min.g). The CL_{int} of BA was also estimated using precision-cut liver slices. The basis for this estimation is in the generation of values for V_{max} and K_m, the ratio then normalized for total protein gives an estimate for CL_{int}. The Cl_{int} was 8.20 ml/min (*in vivo*[3] rat = 10 ml/min). The strategy for such studies, as well as limitations for such experiments, will be presented.

[1]Sprague Dawley; [2]intraperitoneal; [3]Wistar

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200 A STUDY OF THE EFFECTIVENESS OF CHELEX® 100 AS AN ORAL IRON CHELATOR IN REDUCING IRON ABSORPTION IN RATS

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Oral iron overdose is among the leading causes of poisoning in children. There are only a few treatments available for iron toxicity and chelation therapy is one of them. However, there is only one commercially available iron chelator on the market, Deferoxamine, which is given IV or IM. The latter treatment is used only after iron poisoning has been identified and symptoms appear. There is need for an effective oral iron chelator to inhibit systemic absorption of iron following acute oral ingestion of high doses of

iron. In this study we have used a cation-binding chelating resin, Chelex® 100, as an oral iron chelator. The following experiments were done with four male Sprague-Dawley rats per group: 1) 1.5 ml of $FeSO_4$ solution (iron 25 mg) was administered followed 35 minutes later by 1.5 ml buffer; 2) 1.5 ml of $FeSO_4$ solution (iron 25 mg) was administered followed 35 minutes later by Chelex® 100 (1.5g/1.5 ml buffer); 3) 1.5 g of Chelex® 100 was suspended in 1.5 ml of $FeSO_4$ solution (iron 25 mg) and was administered. Plasma samples were analyzed by a colorimetric assay at 562 nm. TCA/HCl and ferrozine were used to precipitate the plasma proteins and to develop the color, respectively. To test if Chelex® 100 inhibited iron absorption the average AUCs of these three groups were compared to each other by the Bonferroni t-test. The resulting mean AUCs ($\pm$ SD), upto 6 hours, for groups 1,2 and 3 were: 14.5 mg.hr/L ($\pm$2.03), 10.9 mg.hr/L ($\pm$2.11) and 14.1 mg.hr/L ($\pm$2.44), respectively. No significant differences were found. These results suggest that iron undergoes rapid desorption from Chelex® 100 in the gut of the animal and is not likely to be an effective inhibitor of iron absorption. Supported by a grant from the Southwest Environmental Health Sciences Center and NIEHS Center Grant (P30E506694).

201 A SIMPLIFIED ISOLATED PERFUSED RAT LIVER APPARATUS (IPRL): EXTRACTION RATIO ESTIMATES FOR ANTIPYRINE

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The liver plays a major role in the biotransformation of various xenobiotics due to the presence of Phase I and Phase II enzymes. In order to better predict *in vivo* events both qualitatively and quantitatively, *in vitro* tools are being widely used. A simple perfusion apparatus has been developed to measure extraction ratios and study drug interactions in the rat liver. A single-pass perfusion system, as opposed to the recirculating system was chosen because it permits the investigation of elimination events only, and the accumulation of metabolites normally seen in the recirculating mode is avoided. A useful indicator of metabolic activity is the metabolism of the drug itself. In order to characterize the system, we tested the hypothesis that the extraction ratio of low clearance compounds would be unchanged with changes in perfusate flow rate. Male Fisher rats (280–310g) were used. The model compound chosen was antipyrine, a low clearance molecule. At an input concentration of 6 mg/L the extraction ratio was studied as a function of varying perfusate flow rates: 15, 20, 25, and 30 ml/min. The extraction ratio was unchanged and ranged from 0.09–0.11. Furthermore, the extraction ratio of antipyrine was studied over 3 hours at a flow rate of 25 ml/min. These extraction ratios agree with experiments performed with other IPRL preparations and predict the *in vivo* hepatic clearance well. In order to ascertain that the liver was receiving sufficient oxygen at a flow rate of 25 ml/min, the extraction ratio of oxygen was studied as a function of flow rate: 15, 20, 25, 30, and 35 ml/min. The extraction ratio decreased from 0.65$\pm$0.1 (15 ml/min) to an average of 0.48$\pm$0.04 (25–35 ml/min) indicating a sufficient supply of oxygen at 25 ml/min. The IPRL appears to be a good tool to estimate extraction ratios and study drug interactions.

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202 PHARMACOKINETICS AND METABOLISM OF THE ENANTIOMERS OF p-HYDROXYMETHAMPHETAMINE (OHMAP) IN RATS

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OHMAP is one of the major metabolites found in humans and rats of the widely abused drug, methamphetamine. In rats, the formation of OHMAP from MAP involves a stereoselective process but its elimination has never been reported. To study the disposition kinetics as well as metabolism of OHMAP, we employed a sensitive HPLC method which can separate the enantiomers of OHMAP and *p*-hydroxyamphetamine (OHAP), one of the metabolic products found in vitro. Both conjugated and unconjugated forms of these compounds can be quantitated. We have previously reported the details of that assay. Male Sprague-Dawley rats were given an iv bolus of racemic OHMAP (20 mg/kg) and serum and urine samples were collected at selected times. The serum concentration-time data for OHMAP enantiomers could be described by a biexponential equation. The clearance (*CL*) of *d*-OHMAP (93.5 ml/min•kg) is significantly greater than that of the *l*-enantiomer (83.9 ml/min•kg). The volume (V_{ss}) of *l*- and *d*-OHMAP are (mean $\pm$ SD) 3.15$\pm$0.84 and 4.23$\pm$1.76, respectively. No significant amounts of OHAP enantiomers could be detected in any sample. Rats excreted more unchanged *l*-OHMAP (34%) than *d*-OHMAP (29%). In contrast, more conjugated *d*-OHMAP (57%) was recovered compared to the conjugated *l*-OHMAP (52%). However, the total % recoveries of both enantiomers are similar ($\approx$ 86%). The results suggest that there is stereoselectivity in the disposition of OHMAP enantiomers. The N-demethylation product (OHAP) was not produced in vivo. Supported by a grant from the National Institute on Drug Abuse (DA 06775).

203 DISPOSITION KINETICS AND DERMAL ABSORPTION OF N,N-DIETHYL-*m*-TOLUAMIDE (DEET)

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DEET is a widely used insect repellent which appears to be absorbed by both animals and humans following dermal application. The toxicokinetic profile of DEET and its interactions with other xenobiotics has not been thouroughly studied. A preliminary study was performed to determine the pharmacokinetics of DEET in male Sprague-Dawley rats. The study provided for comparison of values from both dermal and intravenous routes of administration using the following protocols. An intravenous (IV) 3 mg dose of DEET was administered via a jugular vein catheter. The dose was given in 0.3 ml of a solution containing 40% propylene glycol and 10% ethyl alcohol. Blood samples were obtained over a period of four hours. A solution containing 15 mg of DEET in 0.1 ml acetone was applied dermally to a shaved area on the back. Blood samples were obtained over a period of 48 hours. Plasma samples were treated using liquid-liquid extraction and analyzed for DEET by gas chromatography using a nitrogen-specific detector. Non-compartmental analysis was performed on the IV and dermal data to obtain the kinetic parameters of clearance (CLs), and half-life ($t_{1/2}$). CLs following IV dosing averaged 17.6 ml/min. The terminal half-life averaged 18.7 min. In marked contrast, the concentration-time profile following dermal application suggested slow absorption during the sampling period. Dermal absorption appears to be nearly complete. Elucidation of the kinetics of DEET in rats will be applicable to further studies of its interaction with pyridostigmine when given concomitantly.

204 PHARMACOKINETICS OF ACRYLAMIDE AFTER ORAL EXPOSURE IN MALE RATS

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Acrylamide is a commonly used industrial chemical. However, it produces a dying back type of peripheral neuropathy. This study was performed to investigate the pharmacokinetics of acrylamide after oral administration at (50 mg/kg b.wt. 1-^{14}C-acrylamide) in rats. Absorption from the G.I. tract was rapid. Plasma c_{max} was 46.6 μg/ml and t_{max} was 38 min. The absorption half life of acrylamide in plasma was 13 min, while in the blood was 1.4 hr. The elimination pattern was fitted in one compartment model with 6 hr half life in plasma and 76 hr in blood. The results of tissue distribution studies 28 and 144 hr post-administration were different. After 28 hr the highest activity was in the gastric content, followed by stomach, lung, bone marrow and skin. While after 144 hr the order of the highest total activity was lung > pancreas > adrenal > esophagus. The excretion study revealed that the kidney is the major route of elimination. The feces contained approximately 10% of the administered dose after 144 hr. (Supported by Hazardous Substance Management Research Center/New Jersey Commission on Science and Technology and CYTEC Industries, West Paterson, NJ).

205 TISSUE-TO-PLASMA PARTITION COEFFICIENTS (PCs) FOR HYDROQUINONE (HQ) IN MALE FISCHER AND SPRAGUE-DAWLEY RATS

T S Hill, D A Morgott, and J C English. *Health and Environment Laboratories, Eastman Kodak Company, Rochester, NY*

HQ tissue-to-plasma PCs were measured in male Fischer (F-344) and Sprague-Dawley (SD) rats. PCs were calculated as the ratio of the free HQ fraction measured in tissue and plasma at steady-state. The free fraction, in turn, is dependent upon the degree of binding to proteins. An ultrafiltration device (Amicon Centriprep-10 Concentrators) was used to separate free from bound HQ after the addition of radiolabeled HQ (final concentration of 2–4 μg/g) into liver, kidney, small intestine, and lung homogenates, plasma, and

red blood cells (RBC) suspensions. Samples were maintained on an ice bath to limit metabolism. Separate determination of irreversible binding was measured by repetitive solvent washing of precipitated proteins. The effects of metabolism and homogenate dilution were independently evaluated. Differences between F-344 and SD rats in the amount of free and bound HQ in tissues were small. The extent of irreversible HQ binding measured in plasma, liver, kidney, and lung was 1.55 to 3.01%. The percentage of irreversibly bound HQ was 18.1% ± 2.5 (mean ± sd) in RBC and 9.3% ± 5.4 in the small intestine. The high percentage of irreversible HQ binding in RBC is likely due to nonenzymatic oxidation of HQ inside the RBC. The percentage of unbound HQ in all tissues was 70.4 to 89.3%, whereas the percentages in plasma and RBC were 39.8% ± 1.0 and 49.2% ± 7.8, respectively. The reversible binding of HQ in plasma was 57.6% ± 0.9. The PCs for HQ in tissues were 1.3 to 2.4, with the lowest value occurring in RBC and the highest in small intestine. These findings suggest that constituents of whole blood may act as a reservoir that can affect the overall transport and distribution of HQ to tissues. (Supported by Eastman Chemical Company, Mitsui Petrochemicals (America), Ltd., and Rhone-Poulenc Inc.)

206 POTENTIAL FOR RETINAL ACCUMULATION OF A PHOSPHOROTHIOATE OLIGONUCLEOTIDE (ISIS 2922) AFTER INTRAVITREAL INJECTION IN CYNOMOLGUS MONKEYS

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The kinetics of an intravitreally administered phosphorothioate oligonucleotide designed to inhibit cytomegalovirus replication, ISIS 2922, were investigated in cynomolgus monkeys. Vitreal and retinal concentrations were determined after administering three different doses: 11, 57, and 115 μg/eye. ISIS 2922 concentrations in vitreous and retina were examined after single doses as well as weekly or biweekly doses. ISIS 2922 levels were determined using solid phase extraction followed by capillary gel electrophoresis. Vitreous and retina concentrations of ISIS 2922 were dose proportional and nearly dose linear. The vitreous concentrations three days after dosing range from 40 nM to approximately 1.5 μM. By 15 days after the intravitreal injection the concentrations were below the limit of detection in all dose groups (<10 nM). There was no indication of accumulation in vitreous after multiple weekly or biweekly dosing. The retina concentrations of ISIS 2922 three days after a single intravitreal injection ranged from 50 nM to 1.5 μM, for the 11 and 115 μg doses, respectively. On day 3 after a single 57 μg dose retinas contained 0.71 ± 0.24 μM ISIS 2922 (n=4) and three days after three biweekly doses the retinal concentration of ISIS 2922 was 0.69 ± 0.30 (n=4); while 3 days after the last of 4 doses, three weekly followed by one two weeks later, the retina ISIS 2922 concentration was 1.15 μM (n=2). The results of analyses from the multiple dose study suggested that weekly but not biweekly injections caused accumulation of ISIS 2922 in the retina. The results of the present study will help determine dosing regimens in clinical trials.

207 PLASMA LEVELS AND TISSUE DISTRIBUTION OF A PHOSPHOROTHIOATE OLIGONUCLEOTIDE (ISIS 2302) AFTER INTRAVENOUS ADMINISTRATION

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ISIS 2302 is a phosphorothioate deoxyribo-oligonucleotide currently being investigated as an antisense inhibitor of intercellular adhesion molecule 1. The disposition of ^{14}C-labeled ISIS 2302 was determined in rats following the administration of either a single or 4 IV bolus injections at two dose levels (4 and 20 mg/kg). Intact oligo concentrations were measured by anion-exchange HPLC of proteinase K digested and phenol chloroform extracts of plasma and tissues. After a single dose, plasma levels were 0.66 ± 0.11 μM and 5.9 ± 0.4 μM after 1 hr and declined to 0.009 ± 0.002 μM and 0.23 ± 0.03 μM by 4 hrs at 4 and 20 mg/kg doses, respectively. There was no accumulation of ISIS 2302 in plasma after multiple doses. Higher concentrations of ISIS 2302 were found in kidney and liver compared to heart, spleen, lung, small and large intestine, bone marrow, lymph nodes and skin at both doses. The concentrations of intact ISIS 2302 in kidney were higher than liver at all time points from 1 to 240 hours at both doses after single and multiple administrations. In contrast to plasma, substantial oligo levels were found in liver and kidney up to 240 hrs after a single dose of 20 mg/kg and up to 48 hours after multiple doses. Very low concentrations of ISIS 2302 were found in urine and feces at both doses (<0.05 μM). In summary, ISIS 2302 was distributed rapidly from plasma to tissues, particularly liver and kidney. These findings may be important for the design and interpretation of toxicity studies.

208 THE ABSORPTION AND DISPOSITION KINETICS OF 3,3',4,4'-TETRACHLOROAZOXYBENZENE (TCAOB) IN THE MALE FISCHER 344 RAT

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3,3',4,4'-tetrachloroazoxybenzene (TCAOB) is a structural analog of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). TCAOB is formed as a byproduct during the synthesis of industrial products and exhibits similar toxicity and Ah receptor binding characteristics to those defined for TCDD. In this study, the absorption and disposition kinetics of TCAOB were examined in male F-344 rats. The animals were administered [^{14}C]-TCAOB (3.4 or 34 mg/kg p.o., 3.4 mg/kg i.v.) and the excretion of the radiolabel was monitored over 96 h. Following the low and high dose, 35% and 30% of the [^{14}C] was eliminated in the urine with 55% and 54% eliminated in the feces, respectively. At 96 h, the adipose tissue:blood ratios of [^{14}C]-TCAOB equivalents were 8 and 25 for the oral low and high doses and 21 following the i.v. dose. Other tissue to blood ratios were of little significance (0.06–3.2). Pharmacokinetic parameters indicate that the parent molecule is cleared from blood with an average half life ($t_{1/2}$) of 7 h and an average clearance (CLs) of 11.2 ml/kg(min). Absolute bioavailability was calculated to be about 9%. Metabolite analysis of the urine revealed a variety of dichlorolaniline conjugates, which indicates that azo reduction occurs. Animals dosed with oral antibiotics to eliminate gut microorganism resulted in an increase in the area under the concentration time curve (AUC) following oral administration and a decrease in the AUC following i.v. administration. Therefore, elimination of gut microorganisms alters the kinetics of TCAOB. The nature of the fecal and biliary metabolites are presently under investigation (Supported by NIEHS NO1-ES 35367 and Center Grant P30 ES06694)

209 THE PLASMA PROTEIN BINDING OF HYDROQUINONE (HQ) IN RATS AND HUMANS

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The plasma protein binding of a chemical can influence its distribution, metabolism, and elimination. Studies were conducted to determine the extent to which HQ binds reversibly and irreversibly to plasma proteins. Pooled plasma samples from male Fischer (F-344) rats, male Sprague-Dawley (SD) rats, and female human volunteers were incubated with 2 to 16 μg/g radiolabeled HQ and ultrafiltration devices (Amicon micropartition tubes) were used to separate free from bound HQ. Irreversible binding was determined separately by exhaustive washing of precipitated plasma proteins. The percentage of total bound HQ at 37 °C was (i) 49.3 ± 2.5% (mean ± sd) for F-344 rats, (ii) 44.6 ± 3.3% for SD rats, and (iii) 35.9 ± 5.1% for humans. When studied at 0 °C, the percentage of total bound HQ was 60.9 ± 1.2% for F-344 rats and 59.6 ± 0.3% for SD rats. The data indicate an increase in the percentage of reversible binding with decreasing temperature. The percentage of HQ irreversibly bound to plasma proteins at 37 °C was (i) 26.2 ± 5.5% for F-344 rats, (ii) 23.5 ± 2.1% for SD rats and (iii) 14.5 ± 2.3% for humans. The percentage of irreversibly bound HQ at 0 °C was 2.9 ± 0.1% for F-344 rats and 2.5 ± 0.2% for SD rats. The dramatic decreases in irreversible binding with decreasing temperature suggest that temperature-dependent biotransformation within plasma results in the formation of reactive products capable of binding covalently to plasma proteins. Such biotransformation within plasma may contribute to the rapid elimination kinetics observed for HQ *in vivo*. There were no appreciable differences between F-344 and SD rats in HQ plasma protein binding; however, a probable species difference was observed for rats and humans. (Supported in part by Eastman Chemical Company, Mitsui Petrochemicals (America), Ltd., and Rhone-Poulenc Inc.)

210 DERMAL ABSORPTION AND PHARMACOKINETICS OF ISOPROPANOL IN THE MALE AND FEMALE F-344 RAT

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Isopropanol (IPA), as a 70% (by weight) aqueous solution, was applied under occlusion (4.3 cm^2 exposure area) to the shaved backs of male and female F-344 rats for a period of 4 hr. An IPA blood concentration of approximately 0.2 μm/g of blood for both males and females was attained at 4 hr and decreased rapidly following the removal of IPA. Acetone (ACE) blood levels rose steadily during the 4-hr exposures reaching peak levels of 0.79 μm/g in male rats (4.5 hr) and 1.17 μm/g in female rats (5 hr). First-order elimination half-lives for IPA and ACE were similar for both sexes with mean values of 0.8 hr for IPA and 2.6 hr for ACE. Following I.V. dosing with ^{14}C-IPA, 55% of the dose in males and 50% of the dose in females were eliminated as $^{14}CO_2$ with lesser amounts recovered as expired volatile compounds or in urine. Total recoveries following I.V. dosing were 83% for both males and females. Following a 4-hr dermal exposure to ^{14}C-IPA, 84% and 86% of the applied doses in males and females, respectively, were recovered from the application site whereas total recoveries were 91% and 92%, respectively. Calculated dermal absorption rates, obtained by two independent methods, showed excellent agreement. The values obtained were 0.78 ± 0.03 and 0.85 ± 0.04 mg/cm^2/hr for males and 0.77 ± 0.13 and 0.78 ± 0.16 mg/cm^2/hr for females. Derived permeability coefficients of 1.37 to 1.50 x 10^{-3} cm/hr for males and 1.35 to 1.38 x 10^{-3} cm/hr for females indicate that IPA is rapidly absorbed through rat skin when applied under occlusion. This study was sponsored by the Chemical Manufacturers Association Isopropanol Panel.

211 NEW DEVELOPMENTS AND APPLICATIONS OF DIVERSE EXPERIMENTAL ANIMAL MODELS SELECTED FOR SPECIFIC TOXICOLOGIC RESEARCH NEEDS

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This Workshop is designed to broadly discuss the selection or development and the application of improved animal models for toxicology, based upon biomolecular, cellular and other physiological traits. The proper choice of experimental animal models is a key factor that enables toxicologists to better extrapolate observed effects to humans and other target species. Selecting the most appropriate model also becomes critical as toxicologists further investigate specific cellular and molecular mechanisms or modes of action of toxicants, thereby affording more confidence for ascertaining relevance of toxic responses and risks to health. Several exemplary models exist, which will be described and contrasted with alternative animal models, whereby an appropriate species has been specifically matched to a health issue or toxicological problem. Speakers will discuss the development or selection of specific animal models recently used to investigate: 1) toxicokinetic and clinical relevance of primate, swine, and rodent models for new concerns of methanol neurotoxicity, 2) essential attributes of surrogate species used to evaluate ecotoxicologic effects and risks in wildlife, 3) use of juvenile swine, versus rodents, as improved indicators of heavy metal bioavailability in children, and 4) refined development and use of transgenic animals to better assess pharmacological and toxicological effects of new drugs and environmental carcinogenic risks.

212 SHOULD BENCHMARK DOSES BE USED IN HUMAN HEALTH RISK ASSESSMENTS?

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The pros and cons of using benchmark doses in place of traditional No Observed Adverse Effect Levels (NOAELs) in determining "safe" levels of exposure to toxic substances merit careful consideration by the toxicology community. This methodology, proposed for use in regulatory decisionmaking for non-carcinogenic toxic effects, employs dose-response data to estimate the parameters of a flexible mathematical dose-response model, such as the Weibull model, which in turn is used to derive "benchmark" doses, i.e., conservative lower confidence bounds on the exposure levels associated with fixed levels of response, such as 1% or 5%. These dose estimates often lie below the observable response range.

Specific topics for this roundtable discussion include model selection, appropriateness of data use, statistical properties of benchmark dose estimates, model sensitivity, extent of conservatism, potential alternative approaches, and mechanistic considerations.

213 DRUG METABOLIC ENZYMES IN DEVELOPMENTAL TOXICOLOGY: INTRODUCTION

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Although much is known about the metabolism of environmental toxicants in adult organisms, little information exists on the role of cytochrome P450 (CYP) enzymes during development. The developing organism is remarkably dynamic, presenting a constantly changing metabolic profile as various enzyme systems are activated or repressed. This may explain the markedly different sensitivities to various toxicants that are exhibited throughout the developmental period. The application of molecular biological methods has provided important information on the roles of these enzymes in modulating the response of the developing organism to toxicological exposures. The first talk will focus on the identification and role of CYPs during early organogenesis, particularly on how these enzymes influence the response of the conceptus and early embryo to toxic chemicals. The second presentation will discuss the identification of CYPs expressed during human development, as many of the enzymes present in adults are not expressed in the fetus. The third speaker will discuss the developmental consequences of loss of expression of particular CYP enzymes, focusing on recent studies employing knockout mice to examine the role of CYP1A1 during development. The last two talks will discuss some of the short and long term consequences of *in utero* exposures to toxic chemicals and the role of CYP in modulating the toxic response of the developing organism. The first of these will focus on the role of CYP2E1 in human fetuses during late gestation and the response of this enzyme to inducing agents such as alcohol. The last talk will discuss the role of CYP1A1 in the activation of the Ki-*ras* oncogene following *in utero* exposure to carcinogens as a mechanism for lung tumor formation in a pharmacogenetic mouse model.

214 CYTOCHROME P450-DEPENDENT MONOOXYGENATION OF EMBRYOTOXIC/ TERATOGENIC CHEMICALS IN HUMAN EMBRYONIC TISSUES

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The expression of specific, functional P450 cytochromes in human embryonic tissues during the period of organogenesis was investigated with a combination of biochemical and molecular probes. Regiospecific hydroxylation of R-warfarin at carbon 10 and TAO-inhibitable O-debenzylation of benzyloxyphenoxazone in microsomal fractions of human embryonic hepatic homogenates were attributed primarily to a relatively robust expression of P4503A7 during organogenesis. Between 50–60 days of gestation, the P4503A7 protein and transcript were both readily detectable with Western and Northern blotting respectively but appeared to be restricted to hepatic tissues. Sequencing of cDNA after RT-PCR indicated that the embryonic isoform was identical to that expressed later in fetal hepatic tissues. P4503A5 transcripts were also detected but in very low abundance as compared with P4503A7 mRNA. The α-naphthoflavone inhibitable O-deethylation of ethoxyphenoxazone and ring hydroxylation of 2-acetylaminofluorene in human embryonic hepatic microsomes was attributed to expression of functional P4501A1. This isoform was not detectable with Western blots nor was the corresponding transcript detected with Northern blots in human embryonic tissues. PCR amplification, cloning and sequencing of the reverse transcribed cDNA provided evidence that the P4501A1 mRNA expressed in human embryonic tissues was identical to that expressed in human adult tissues. Supported by NIH grant ES 04041.

215 COMPARISON OF CYTOCHROME P450 ENZYMES AND THEIR ACTIVITIES IN HUMAN FETAL AND ADULT LIVER

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Cytochrome P450 (P450) enzymes are major catalysts involved in the bioactivation and detoxication of chemicals, particularly in the liver. Although the P450s in adult human liver have been extensively characterized, less is known about these enzymes in fetal tissues and in neonatal and adolescent liver. Immunoblotting and mRNA studies indicate that P450s 1A1 and 3A7 are expressed in human fetal liver. However, P450s 1A2, 2A6, 2C (several P450s), 2E1, and 3A4 have not been detected. These differences have implications for the fetal disposition of potentially toxic chemicals, including drugs. The patterns of catalytic activities in fetal liver reflect the enzyme composition mentioned. The activation of the food mutagen Trp-P-1 (3-amino-1, 4-dimethyl-5*H*-pyrido[4,3-*b*indole) was detected, possibly due to the presence of P450 1A1. An interesting observation is that the activation of both (+) and (-)-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene is actually higher in fetal liver than adult liver. This activity was not inhibited by 7,8-benzoflavone or anti-P450 1A1 antibodies and does not appear to be attributable to P450 1A1. Another fetal P450 may play a role in this activity. A better understanding of the P450 enzymes in fetal tissues may lead to insight in appropriate drug regimens and risks of chemicals in the environment. (Supported in part by USPHS grants CA44353 and ES00267 and grants from the Ministry of Education, Science, and Culture of Japan and Osaka Prefectural Government)

216 USE OF KNOCKOUT MOUSE LINES TO STUDY THE ROLE OF DRUG-METABOLIZING ENZYMES DURING REPRODUCTION AND DEVELOPMENT

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The dioxin-inducible murine [*Ah*] battery contains at least six genes and is believed to play an important role in reproduction, development, and oxidative stress. In addition to two P450 genes—***Cyp1a1*** and *Cyp1a2*—this laboratory has shown that four Phase II [*Ah*] genes include: NAD(P)H: menadione oxidoreductase (*Nmo1*); a cytosolic "class 3" aldehyde dehydrogenase (***Ahd4***); a UDP glucuronosyltransferase having 4-methylumbelliferone as substrate (***Ugt1*06***); and a glutathione transferase having 2,4-dinitro-1-chlorobenzene as substrate (***Gsta1***, Ya). The Ah receptor (***Ahr***)-mediated coordinate induction is controlled positively and involves all six [*Ah*] battery genes. This laboratory is generating conventional, plus inducible, knockout mouse lines having homozygous disruptions in the above-mentioned genes. If the conventional knockout is lethal during development, this model would be important for developmental biology, and the inducible knockout can still be used for studies involving the mechanisms of environmental toxicity and cancer. ***—Supported in part by NIH Grants R01 AG09235, R01 ES06321, R01 ES06811, and P30 ES06096.***

217 IDENTIFICATION AND CHARACTERIZATION OF HUMAN FETAL CYP2E1

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Despite proposed mechanistic theories regarding ethanol-mediated teratogenesis, a definitive pathogenesis has not been elucidated. Formation of the teratogen acetaldehyde or alcohol radicals by fetal ethanol oxidizing enzymes are possible etiologies. Therefore, we examined whether the alcohol inducible P450, CYP2E1 (2E1), is expressed in human fetal liver. Liver samples from fetuses ranging in gestational age from 18–24 weeks were examined for 2E1 content. Immunoblot analysis demonstrated a fetal hepatic microsomal protein that migrated slightly faster than 2E1 present in adult microsomes. Further confirmation of 2E1 expression was demonstrated in hepatic RNA from a 19 week gestation fetus by reverse transcriptase-polymerase chain reaction. 2E1 mRNA was shown by RNase protection assays to be expressed only in liver samples from latter gestational ages. Fetal 2E1 catalytic properties were assessed by their ability to oxidize ethanol and oxidation rates were found to be 12–27 % those of adult liver microsomes. Immunoinhibition studies confirmed that the major catalyst was 2E1. Xenobiotic-mediated inducibility of fetal 2E1 was also investigated. Isolated fetal hepatocytes treated with ethanol or clofibrate, demonstrated a 2-fold 2E1 increase compared to untreated hepatocytes. These results indicate that 2E1 is present in human fetal liver, it is catalytically similar to its adult counterpart, and xenobiotics including ethanol and clofibrate induce the enzyme *in vitro*. Fetal 2E1-mediated ethanol metabolism may contribute to the local production of free radicals and acetaldehyde, reactive species that could potentially produce deleterious effects on the human fetus. Supported by AA08990.

218 FREQUENT MUTATIONS IN Ki-*ras* IN MOUSE LUNG TUMORS FOLLOWING TRANSPLACENTAL EXPOSURE TO 3-METHYLCHOLANTHRENE

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A pharmacogenetic mouse model was utilized to study the role of *Cyp1a1* in modulating damage to the Ki-*ras* gene. *Ah* responsive male B6D2F1 mice were mated with nonresponsive DBA female mice and the pregnant mothers treated on the 17th day of gestation with a single ip injection of 30 mg/kg of 3-methylcholanthrene (MC). Thus, both responsive and nonresponsive fetuses were residing within the same maternal environment during *in utero* exposure to MC. The lung tumor incidence in responsive male and female offspring was 93% and 92%, respectively, compared to their nonresponsive littermates, which exhibited lung tumor incidences of 39% and 50%, respectively. Paraffin-embedded lung tumor tissue was used to amplify exons 1 and 2 from the Ki-*ras* gene. The Ki-*ras* PCR products were screened by allele-specific oligonucleotide hybridization. Thirteen of 16 tumors (81%) exhibited mutations in the 12th or 13th codon, with 7 tumors showing GGT - GTT (GLY^{12} VAL^{12}), 4 exhibiting GGTraTGT (GLY^{12}raCYS^{12}), and 2 harboring GGCraCGC (GLY^{13}raARG^{13}) mutations. None of the tumors had mutations at the 61st codon. The PCR products from 4 of the amplified tumor samples were cloned and the results confirmed by sequence analysis. The predominance of GraT transversions would be expected as a result of MC binding to guanine bases in the DNA, and is the predominant lesion observed in human adenocarcinomas. Despite the resistance of these strains to chemically-induced cancers when treated as adults, the offspring had a high incidence of lung tumors with a mutant Ki-*ras* gene. These results demonstrate the high susceptibility of the fetus to carcinogenic chemicals.
(Supported by NIH grant ES06501).

219 BRAIN METALLOTHIONEINS - THE ROLE IN PHYSIOLOGY AND PATHOLOGY

Chairperson: Michael Aschner. *Bowman Gray School of Medicine, Medical Center Blvd., Winston-Salem, NC*

The objective of this symposium is to define the role of brain metallothioneins (MTs) both in brain physiology and pathology. MTs are small, cysteine-rich, metal-binding proteins that are expressed in all eukaryotes that have been examined. In mammals, 2 isoforms, MT-I and MT-II, are expressed in most organs and they are regulated by a number of metals, hormones, and xenobiotics. Recent studies have revealed a brain-specific isomer, MT-III. Proposed functions attributed to brain MTs include detoxification of metals and their storage, zinc storage for the demands of cell differentiation and proliferation, metal transfer, and scavenging of free radicals. The existence of a brain-specific isoform of MT-III also suggests that it might have important neurophysiological functions, especially in the utilization of zinc as a neuromodulator. This idea is reinforced by the potential involvement of MT-III in a number of neurodegenerative disorder, and its depletion in humans afflicted by Alzheimer's disease, and recent observations on the increased sensitivity of MT-III "knockout" mice to kainate-induced seizures. The specific aims of this symposium are: 1) to review the physiologic functions and biochemical properties of MTs, 2) examine the distribution of brain MT with particular emphasis on cell-specific localization (neurons vs. glia), 3) to discuss MT gene responsiveness upon brain injury, and 4) to discuss the potential role of MTs in the etiology of neurodegenerative disorders.

220 BIOCHEMICAL PROPERTIES OF METALLOTHIONEINS

M.George Cherian. *Department of Pathology, University of Western Ontario, London, CANADA*

Metallothioneins (MT) are intracellular metalloproteins of low molecular weight (6000 Da) with a high cysteine content (33%) and a high affinity for

certain metals. The synthesis of these proteins can be induced by metals and other chemicals. Both the solution and crystal structure of MT show two distinct adamantane-like metal binding clusters. Four major isoforms of MT have been isolated from mammals. In mouse, the MT gene family is found in chromosome 8 while in human they are localized in chromosome 16. Both MT-1 and 2 genes are expressed in various organs while MT-3 is expressed only in brain. The expression of MT-4 is also restricted to cornified and stratified squamous epithelium of skin, tongue and upper part of alimentary tract. The biological functions of these proteins are still unknown but recent studies with transgenic mice show that they have a role in protecting animals from cadmium toxicity. The brain-specific MT-3 is also known as growth inhibitory factor because it can inhibit neurotrophic activity. It is structurally different from MT-1 and 2 and contains 8 additional amino acids. MT-3 is mainly localized in certain neurons and glia. In addition to MT-3, brain also contains MT-1 and 2 isoforms. Immunohistochemically these two isoforms are localized in astrocytes but not in oligodendroglia and microglia. In the astrocytes, MT may be involved in the cellular defence mechanism against reactive organic intermediates and free radicals. However, the induction of MT-3 synthesis has not been demonstrated, after exposure to chemicals.

221 EXPRESSION OF METALLOTHIONEIN GENES IN BRAIN

C D Klaassen, S Choudhuri, K K Kramer and H Zheng. *University of Kansas Medical Center, Kansas City, KS*

Three isoforms of metallothionein (MT) are known to be expressed in brain tissue. Constitutive expression of the three MT mRNAs in whole mouse brain was similar. Seven different brain regions investigated in this study also had similar levels of the three MT mRNAs. *In situ* hybridization revealed: (1) MT-1 mRNA was predominantly localized in glial cells of the Purkinje layer of the cerebellum and also in the ependymal cells of the lateral ventricles, while (2) MT-III mRNA was preferentially localized in the granular layer of the dentate gyrus. Studies on the regulation of MT-I and III mRNAs in brain by *in situ* hybridization following treatment by known MT inducers revealed: (1) that MT inducers appeared to enhance MT-I mRNA in the Purkinje layer of the cerebellum, and (2) MT-III mRNA was reduced in hippocampus by kainic acid (KA), dexamethasone (Dex) and lipopolysaccharide, while MT-III was increased in cortical regions by KA, Cd and ethanol treatment. The profile of changes in MT-I and -III mRNA expression observed in mouse brain following treatment by various inducers, was reproducible in studies with cultured astrocytes and neurons. In cultured astrocytes, Cd, Zn, Dex and Hg increased MT-I and MT-II mRNA expression while MT-III was unaffected. These same inducers increased MT-I and -II, but decreased MT-III mRNA expression by 50% in neurons. Thus, the differing regulation and cellular distribution of MT-I and -III in brain suggests specific, but yet, unknown functions for MT isoforms. (Supported by NIH grant ES-01142)

222 HEAVY METAL AND pH INDUCED METALLOTHIONEIN EXPRESSION IN ASTROCYTES

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Astrocyte dysfunction and the ensuing changes in the composition of the extracellular microenvironment may be a common etiologic factor in neurodegeneration. To explore the possibility that astrocytic MTs afford a protective role, we investigated the effects of a number of heavy metals and acidic pH on the induction of MTs both at the mRNA and protein level. MT-I and MT-II mRNA were detected in untreated cells, suggesting constitutive MT expression in astrocytes. Methylmercury (MeHg), Cd, Bi, and Zn, as well as acidic pH increased MT-I and MT-II mRNA levels over controls. Western-blot analysis revealed a time-dependent increase in MT protein synthesis through 96 hours of exposure to all treatments. The toxic effects of MeHg were assessed by the rate of astrocytic [^{3}H]-D-aspartate uptake, and inhibition of the regulatory volume decrease (RVD) in astrocytes swollen by means of hypotonic solution. Induction of MTs by exposure to $CdCl_2$, completely reversed the inhibitory effect of MeHg on [^{3}H]-D-aspartate uptake and RVD which occur in MeHg-treated astrocytes with constitutive MT levels. In summary, (1) treatment with a number of heavy metals, as well as acidic pH increase astrocytic MT expression, and (2) increased MT levels attenuate MeHg-induced astrocyte toxicity. Increased MT expression in astrocytes may represent a generalized response to "stressors", protecting both astrocytes and indirectly juxtaposed neurons. (Supported by NIH ES-05223 and EPA 89190).

223 PROPERTIES OF METALLOTHIONEIN-III (MT-III)

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MT-III is expressed predominantly in glutamatergic neurons that sequester zinc in their synaptic vesicles. The neurons of the dentate gyrus and hippocampus are especially rich in MT-III. Transgenic mice that over-express human MT-III by ~10-fold or fail to express MT-III due to gene inactivation, have brain zinc levels that are elevated ~50% or reduced ~10%, respectively. However, vesicular zinc as assessed by neo-Timm's reaction or the fluorescent probe, TSQ, were unchanged. Spatial learning by these mice in a Morris water maze appeared normal. There were also no differences in conditioned learning paradigms. However, the MT-III knockout mice were very sensitive to kainate-induced seizures. They convulsed sooner and more often than littermate controls and there was increased mortality of the MT-III -/- mice. In vitro assays confirmed previously published results that MT-III (but not MT-I or MT-II) inhibits survival of rat neurons cultured in Alzheimer brain extract. This activity depended on the N-terminal β-domain of MT-III and the 2 prolines at positions 7 & 9 were critical for this activity. Another assay revealed that constitutive expression of MT-III (but not MT-I) inhibited growth of BHK cells when they became Zn-deficient. When cells became Zn-deficient MT-I protein disappeared whereas MT-III protein persisted, even though mRNAs for both were abundant. We conclude that MT-III has unique properties but we cannot yet explain how it protects against seizures.

224 CEREBRAL ZINC METABOLISM: PATHOGENIC POTENTIAL IN ALZHEIMER'S DISEASE

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Aβ the principal component of cerebral amyloid collections characterizing Alzheimer's disease (AD), is a ≈40 residue peptide found in cerebrospinal fluid but levels are not raised in the sporadic disorder. We systematically appraised neurochemical factors that may interact with Aβ to promote amyloid formation and showed that the peptide specifically and saturably binds zinc, manifesting both high-affinity binding (K = 107 nM) compatible with normal cerebrospinal fluid (CSF) zinc levels, and low-affinity binding (K = 5.2 μM) (Bush *et al.*, J. Biol. Chem, 269, 12152; 1994). Zinc binding increases the peptide's adhesive properties, resistance to proteolytic digestion, and at >300 nM, rapidly destabilizes human Aβ solutions, producing 0.65–40 μm particles in <2 minutes. The rat species of Aβ is resistant to these effects, consistent with the scarcity with which these animals form cerebral Aβ amyloid (Bush *et al.*, Science 265, 1464; 1994). Although Fe(II) and Cu(II) are capable of inducing some aggregation, only Zn(II) induces a readily sedimentable, relatively large (10–40 μm), congophilic reaction product (Bush *et al.*, Science 268, 1921; 1995).

Evidence for altered zinc metabolism in AD includes decreased temporal lobe zinc levels, increased hepatic zinc with reduced zinc bound to metallothionein (MT), and increase in extracellular Zn(II)-metalloproteinase activities in hippocampus, and decreased brain levels of zinc-chelating MTIII (a MT expressed in neurons that sequester zinc in synaptic vesicles). The distribution of MT isoforms correlates with the neuroanatomical distribution of plaque amyloid. Collectively, these reports indicate that there may be an abnormality in the uptake or distribution of zinc in the AD brain. A pervasive abnormality of zinc metabolism has been described as a common complication of Down syndrome, a condition invariably complicated by premature AD. Indeed, therapy with desferrioxamine, a drug which chelates zinc, has been reported as effective in arresting the clinical progression of AD. We are currently assessing the interaction of cerebral zinc metabolism with Aβ *in vivo*, as a putative upstream lesion in the pathogenic cascade of AD.

225 DIFFERENTIAL LARGE PARTICLE CLEARANCE BY MOUSE AND RAT ALVEOLAR MACROPHAGES *IN VIVO*

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Previous chronic inhalation studies in rats with high inhaled concentrations of particles of low toxicity and low solubility resulted in significant adverse

effects, including impaired particle clearance, pulmonary inflammation and fibrosis and tumors. It has been suggested that a high volumetric particle burden of alveolar macrophages (AM) retards their clearance function resulting in increased pulmonary particle accumulation (volumetric particle overload). A high pulmonary particle load in mice also resulted in impaired particle clearance with pulmonary retention halftimes of up to 1000 days, yet this was not associated with lung tumors and rarely with fibrotic responses in this species. In order to test the volumetric overload hypothesis in mice, we deposited a low mass (~4 μg) of ^{85}Sr and ^{153}Gd-labelled 3 and 10 μm latex spheres in their lungs by intratracheal instillation and determined their clearance subsequently over a period of up to 180 days. In contrast to results of a similar experiment in rats, we found that the large 10 μm particles in mice were still efficiently cleared: The pulmonary retention halftime was ~40 days for the 3 μm particles and ~100 days for the 10μm particles compared to ~60 days (3 μm) and ~1000 days (10 μm) in rats. To further analyze this surprising result, we examined lung lavage samples and found that the large 10 μm particles had not been phagocytized by the mouse AM - in contrast to the rat. Instead, the 10 μm particles were surrounded by a number of mouse AM which resulted in their elimination from the lung. The smaller 3 μm particles had been readily phagocytized by mouse AM. We conclude that (*i*) larger particles can be eliminated from the mouse lung even without being phagocytized and (*ii*) mouse and rat AM behave differently with respect to uptake of large particles, probably due to their different cell size.

226 ACCUMULATION AND PERSISTENCE OF N-METHYLPURINES IN RESPIRATORY TISSUE DNA FOLLOWING REPEATED INHALATION EXPOSURE TO DIMETHYL SULFATE

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Dimethyl sulfate (DMS) is an industrial methylating agent widely used in the preparation of a variety of intermediates and products in the dye, agricultural, pharmaceutical and perfumery industries. DMS has been shown to produce tumors in laboratory animals and is a suspected human carcinogen. Rats were exposed 6h/day for 1, 2, 3, 4, 5 and 10 consecutive days to 0.0, 0.1, 0.7, and 1.5 ppm DMS. The accumulation and persistence of N7 and N3 methylated purines were determined in neutral thermal hydrolysates of DNA isolated from respiratory, olfactory and lung tissues by HPLC using fluorescence and UV detection. DNA adduct formation showed regional differences characteristic of the absorption of a water soluble vapor. Methylation was greatest in DNA isolated from respiratory mucosa, less in olfactory, and little found in lung. Measurements of the accumulation of N7-methylguanine in respiratory mucosa indicated that the kinetics of DMS methylation approached steady state levels by the end of 5 exposure days and decreased by 10 days of exposure. In additional studies, N methylpurine persistence measured in rats exposed 6h/day for 5 consecutive days and measured 1, 2, 3, 6 and 11 days post-exposure appeared to be equilvalent to that observed following an acute 20 min exposure to DMS at 8 or 22 ppm. DMS produced significant histopathological changes following inhalation exposure and epithelial cytotoxicity could be contributing to the rate of loss of methyl purines. These data provide a basis for the development of dosimetry models to be used for interspecies risk extrapolation.

227 SPECIES DIFFERENCES IN NASAL METABOLISM AND UPTAKE OF ACETALDEHYDE VAPOR

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To examine potential species differences in nasal disposition of acetaldehyde vapor, in vitro metabolism kinetics were measured in nasal homogenates of the Sprague-Dawley rat, Syrian hamster, and Hartley guinea pig. In addition, uptake of inspired acetaldehyde vapor was measured in the surgically isolated nasal cavity of these species over a range of inspired concentrations (1–1000 ppm). In all three species, a high affinity-low capacity (Km < 0.1mM) NAD-dependent metabolic pathway for acetaldehyde was detected. A low affinity-high capacity (Km > 10 mM) pathway was detected in nasal tissues of the hamster and rat, but not the guinea pig. In all species nasal acetaldehyde uptake was roughly 2-fold more efficient at low, environmentally or occupationally relevant concentrations (10 ppm or less), compared to that at high concentrations (1000 ppm). Species differences in uptake were observed. Specifically, at 1000 ppm, nasal uptake was most efficient in the rat followed by the hamster and least efficient in the guinea pig. The low uptake in the guinea pig at the high concentration may relate to the lack of the low affinity metabolic pathway. In contrast, at lower exposure concentrations (10 ppm or less), uptake was greatest in the hamster followed by the guinea pig and rat. These studies indicate the complexities of nasal dosimetric relationships and highlight that relationships observed at high exposure concentrations may not mirror those at lower, more environmentally relevant levels. (Supported by NIH ES03676)

228 AN EXPOSURE SYSTEM FOR MEASURING NASAL AND LUNG UPTAKE OF VAPORS IN RATS

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Determining safe exposure levels of gases and vapors for humans relies heavily on data obtained by exposing animals, but predicting safe levels from toxicity studies conducted using animals requires methods to calculate relative doses of vapors and gases to specific sites in the respiratory tract of both experimental animals and humans. In addition, because humans are usually exposed to complex mixtures of gases and vapors, methods to calculate doses of individual components in a gaseous mixture are needed to identify which components are likely to cause site-selective toxicity within the respiratory tract. We have developed a system for accurately measuring the uptake of gases and vapors in the nose and lungs of rats, an animal commonly used in inhalation toxicity studies. Small volumes of breathing zone and tracheal air are automatically sampled during inhalation and exhalation. Vapor concentrations are automatically determined by gas chromotography. Sampling flow rates are matched with respiratory sampling flow rates, which are selectable, allowing effects of different breathing patterns on vapor uptake to be studied. Data generated by this system will be used to validate a mathematical model relating properties of vapors and gases to their dose to specific sites within the respiratory tract; the dose can then be related to observed lesions from inhalation toxicity studies. [Research supported by NIH Grant 4422 from NIEHS in facilities provided by the U.S. DOE/OHER under contract DE-AC04-76EV01013.]

229 CO-EXPOSURE TO MIXTURES OF PARTICLES, SULFUR DIOXIDE, AND OZONE: ALVEOLAR MACROPHAGE EFFECTS

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Three simultaneously occurring air pollutants, particles, sulfur dioxide (SO_2), and ozone (O_3) were co-generated in a flow-past nose-only inhalation chamber. Mice were exposed to components of the mixture for 4 hr and alveolar macrophage (AM) phagocytosis measured a day thereafter. Exposure to 10 mg/m^3 of respirable carbon black particles (CB) or 10 ppm SO_2 at high (>85%) relative humidity (RH) had no effect on AM phagocytosis. Generation of the mixture resulted in formation of particle-associated sulfate (pSO_4^{-2}; 13.7 ± 0.4 μg SO_4^{-2}/mgCB) which, upon exposure, suppressed AM phagocytosis to 74.3% ± 1.0 of air-exposed controls ($p<0.005$). The addition of O_3 at 0.1, 0.3, or 0.5 ppm to the CB and SO_2 mixture increased pSO_4^{-2} levels to 21.6 ±0.7, 29.5 ± 1.1, and 75 ± 2.1 μg SO_4^{-2}/mg CB, respectively. Exposure to the three component mixture continued to suppress AM phagocytosis to 62.4% ± 1.7 at 0.5 ppm O_3 ($p<0.05$ vs the CB and SO_2 mixture). These data demonstrate that CB acted as a vector for the delivery of pSO_4^{-2} to the distal lung which impairs of AM function. The presence of O_3 in the co-generation mixture increased pSO_4^{-2} levels on the CB surface which further decreased AM phagocytosis. [Partially Supported By NIEHS Training Grant ES07141]

230 LOSS OF TURBINATE BONE IN THE NASAL AIRWAYS OF RATS AFTER LONG-TERM EXPOSURE TO OZONE

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As part of the Health Effects Institute/National Toxicology Program collaborative study of the health effects of chronic ozone exposure, it was observed that rats chronically exposed to ozone had marked alterations in the nasal airways, including atrophy of the nasal turbinates. The purpose of the present study was to morphometrically assess the severity of the ozone-induced changes in the bony tissue of the maxilloturbinates in these male and female rats. F344 rats (25 males and 102 females) were exposed to 0, 0.12, 0.5, or 1.0 ppm ozone, 6 h/day, 5 days/week for 24 months. Rats were sacrificed

one week after the end of the exposures, and nasal tissues were processed for light microscopy. Amounts of turbinate bone, surface epithelium, and lamina propria were determined using image analysis and standard morphometric techniques. Both male and female rats exposed to 0.5 and 1.0 ppm ozone, but not 0.25 ppm, had significant changes when compared to controls. Ozone-exposed rats had marked bony atrophy, epithelial hyperplasia/metaplasia, and atrophy of the lamina propria. Females exposed to 0.5 and 1.0 ppm ozone had 5% and 38% less bone (atrophy), respectively, compared to air-exposed controls. Males had even greater reductions in bone after exposure to 0.5 and 1.0 ppm ozone (49% and 52%, respectively). In addition, these ozone-exposed male and female rats had conspicuous chronic active inflammation (rhinitis). The results of the present study demonstrated that chronic ozone exposure causes marked loss of turbinate bone. The severity of the bony atrophy was concentration-and gender-dependent. Research supported by the Health Effects Institute under research contract 94–1.

231 EFFECTS OF 9 MONTHS EPISODIC EXPOSURE TO NITRIC ACID AND OZONE ALONE AND IN COMBINATION ON PULMONARY STRUCTURE AND FUNCTION IN THE RAT

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Fischer-344 rats were exposed nose-only to purified air, 50 $\mu g/m^3$ nitric acid (HNO_3) vapor, 0.15 ppm ozone (O_3), and a combination $O_3 + HNO_3$ for 4 h/day, 3 d/week for 9 months (40 weeks). Biological effects on the respiratory system were analyzed following 9 months of exposure and, for some measures, following 3 months of exposure. At 9 months, the permeability of the nasal epithelium was significantly elevated in HNO_3 and $HNO_3 + O_3$ (main effect HNO_3 $p<0.01$). Pulmonary macrophages collected by bronchoalveolar lavage (BAL) at both 3 and 9 months exposure showed a trend toward reduced phagocytosis of polystyrene latex particles in the combined $HNO_3 + O_3$ exposure but not in individual HNO_3 or O_3 exposures. β-glucuronidase in BAL fluid was significantly elevated ($p<0.05$) in the combination exposure at 9 months and showed a trend of increase at 3 months. Morphometric analysis of lung tissues at 9 months exposure did not show major alterations in any exposure group. However, trapped gas volume in excised lungs after 3 months exposure was increased as a main effect of HNO_3 ($p<0.001$) and of O_3 ($p<0.05$). At 9 months there remained a trend of increased trapped gas volume in all exposure groups. Quasi-static compliance showed a significant depression as a main effect of O_3 ($p< 0.01$) at 3 months, but no significant effect at 9 months. Following 9 months of exposure, all exposure groups had a significant increase in lung heat shock protein 70 levels ($p< 0.01$). At the concentrations tested, the biological effects of long term exposure to HNO_3 and O_3 were generally small, and there was not an indication that HNO_3 and O_3 interacted synergistically. The relationships among these biological effects suggest that exposure to low concentrations of HNO_3 and O_3 may depress defenses against respiratory tract infections and induce some changes in elasticity and fine structure of the lung. Supported by California Air Resources Board AO33-088.

232 CIGARETTE SMOKE-INDUCED ALTERATION OF SURFACTANT SECRETION BY TYPE II CELLS

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We have previously shown that chronic exposure to cigarette smoke causes a significant reduction in the amount of surfactant in bronchoalveolar lavage fluids but not in lung tissues of exposed rats (*FAAT 27: 63, 1995*). To determine if an inhibition of surfactant secretion by Type-II cells is responsible for this reduction, we compared the *in vitro* secretion of phosphatidylcholine (PC) by type II cells isolated from lungs of room control (RC) and cigarette smoke-exposed (SM) rats, in the presence and absence of secretagogues. The basal PC secretion was significantly reduced (25%) in SM group as compared to RC. However, ATP-stimulated secretion of PC was greater in SM than RC group (212 ± 10 vs 174 ± 2%, $P < 0.05$) and the increase was eliminated by pretreatment of cells with H-7, an inhibitor of protein kinase C (PKC). Phorbol ester (TPA), an activator of PKC, increased PC secretion to a greater extent in SM than RC group (233 ± 14 vs 198 ± 13%), while ionomycin and (N-ethylcarboxyamido)adenosine-stimulated secretion remained the same in RC and SM groups. Synthesis of surfactant in type II cells, as measured by incorporation of ^{14}C-choline, was not different in RC and SM groups, and was not affected by secretagogues. These results suggest that cigarette smoke reduces basal surfactant secretion and increases the ATP-stimulated PC secretion by activation of PKC (KTRB 5-41110).

233 INHIBITION OF KUPFFER CELL PHAGOCYTIC FUNCTION POTENTIATES LIPOPOLYSACCHARIDE-INDUCED ALVEOLITIS

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Kupffer cells (KCs) are the resident tissue macrophages of the liver and remove lipopolysaccharide (LPS), derived from gram negative bacteria, from the portal circulation. Inhibition of KC-mediated phagocytosis may predispose distal organs, such as the lung, to LPS-induced inflammation and tissue injury. Female, Sprague-Dawley rats (180–225 g) were pretreated with either $GdCl_3$-$6H_2O$ (10 mg/kg, iv) or saline 24 hr prior to receiving either LPS (4 mg/kg, iv) or saline vehicle. Rats were killed at 1.5, 6, and 24 hr post-LPS to assess inflammatory changes. In a preliminary study, exposure to $GdCl_3$ decreased both clearance of colloidal carbon from blood and uptake of carbon in the liver, indicating inhibition of KC phagocytosis. LPS administration resulted in accumulation of neutrophils (PMNs) in the pulmonary tissue at 1.5 and 6 hr that was unaffected by $GdCl_3$ pretreatment. Exposure to $GdCl_3$ prior to LPS administration resulted in a significant increase in the number of PMNs recovered by bronchoalveolar lavage at 24 hr, indicating diffuse acute alveolitis. Exposure to either saline or $GdCl_3$ in the absence of LPS did not produce alterations in lavage fluid content or pulmonary inflammation. These results suggest that KCs play a role in regulating the migration of PMNs to the airways of the lungs following systemic exposure to LPS. (Supported by NIH grant ES05722)

234 EFFECT OF CHRONIC CIGARETTE SMOKE INHALATION ON THE DISTRIBUTION OF $^{239}PuO_2$ PARTICLES IN RAT LUNGS

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To understand how combined exposures might influence lung cancer risks, rats were acutely exposed to $^{239}PuO_2$ and/or chronically to cigarette smoke (CS) for their life spans. Preliminary conclusions are that (1) these agents interacted synergistically in causing lung tumors, and (2) CS enhanced the lung retention of Pu. Rats were exposed chronically to CS at a concentration of 250 mg total particulate matter/m^3 for 6 hr/day, 5 d/wk, beginning at 6 wk of age, and once to $^{239}PuO_2$ (~400 Bq lung burden) at 12 wk of age. To better understand the results of this chronic study, we used light microscopic autoradiography to localize $^{239}PuO_2$ particles at 2, 10, and 50 wk after $^{239}PuO_2$ exposure within the constant pressure-fixed lungs of two female rats per time exposed to Pu-alone or to Pu + CS. Particles were assigned to a specific anatomic site using a hierarchal morphological classification system. The number of particles correlated well with the radiochemically determined Pu content of lungs in both groups. At all three evaluation times, > 80% of particles in lungs from both groups were within alveolar macrophages, and a greater percentage of particle aggregates were found in the Pu + CS group compared to the Pu-only group. By 50 wk after $^{239}PuO_2$ exposure, 93% of the particles and all aggregated particles in the lungs of the Pu + CS-exposed rats were found in areas of alveolitis, typified by large numbers of lumenal macrophages and lesser numbers of neutrophils. Results demonstrate an association between CS-induced, enhanced particle retention and particle aggregation and sequestration in macrophages within CS-induced alveolitis. [Research supported by the Assistant Secretary for Defense Programs, U. S. Department of Energy, under Contract No. DE-AC04-76EV01013.]

235 NEUROENDOCRINE CELL EXPRESSION IN FETAL LUNGS AFTER MATERNAL EXPOSURE TO AGED AND DILUTED SIDESTREAM CIGARETTE SMOKE (ADSS)

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Pulmonary neuroendocrine cells (PNEC) are specialized airway epithelial cells that may be involved in chemoreception and cellular differentiation. These cells are most abundant during the fetal and neonatal stages of development. The purpose of this study was to examine the effects of maternal exposure to ADSS on the expression of PNEC. Timed-pregnant Sprague-Dawley rats were exposed to ADSS (1mg/m^3, total suspended particulates) for 6 hours/day, 7 days/week, from gestational day (DGA) 5 to the day prior

to sacrifice, while control mothers were exposed to filtered air only. Calcitonin gene-related peptide (CGRP), a neuroendocrine marker, was used to visualize PNEC immunohistochemically in lung tissues at 14, 18 and 21 DGA. The frequency of positive cells in the airways was measured per mm of basal lamina. At 14 DGA, CGRP positive cells were not detected in the airway epithelium of either group. At 18 DGA, CGRP positive cells were found to be in greater abundance in central airways compared with peripheral airways. Maternal exposure to ADSS resulted in a significant increase in CGRP positive cells at 18 DGA within central (3.29 ± 0.35 vs. control, 1.92 ± 0.18) and peripheral (1.5 ± 0.02 vs. control, 0.56 ± 0.23) airways. These differences were also found at 21 DGA in central (2.03 ± 0.04 vs. control, 1.38 ± 0.16) and peripheral (2.47 ± 0.21 vs. control, 1.53 ± 0.23) airways. We conclude that PNEC differentiation in the fetal lung is site-specific and that maternal exposure to ADSS at environmentally relevant concentrations significantly increases the number of neuroendocrine cells within airways during cellular differentiation of the fetal lung.

236 DIFFERENCES IN DOSIMETRY OF THE CARCINOGENS BENZO(A)PYRENE AND NNK IN AIRWAY MUCOSA

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A diffusion model predicts that highly lipophilic toxicants penetrate the comparatively thick epithelium of the conducting airways much more slowly than less lipophilic toxicants. To validate this model, we sprayed the tracheal walls of a Beagle dog with very small quantities of tritiated highly lipophilic benzo(a)pyrene (BaP), moderately lipophilic pyrene, or slightlylipophilic4-(methylnitrosamino)-1-(3pyridyl)-1-butanone(NNK). We measured the concentration of the hydrocarbons and their metabolites in the circulating blood for up to 6 hr and determined tissue retention at the end of the experiment. Differences in absorption of these compounds into blood were manifested in several independent measurements: The more lipophilic the toxicant, 1) the slower its penetration into azygous vein blood, the principal drainage system from the exposed area of the trachea; 2) the slower its appearance in the systemic circulation; and 3) the more it was retained in the tracheal tissues at the end of the measurement period. Increased retention time in the airway mucosa allowed a greater fraction of lipophilic toxicants to be metabolized locally in the airway walls. This finding led us to conclude that, for example, if the carcinogens BaP and NNK are deposited at the same surface density on the airway mucosa, the highly lipophilic BaP will reach a far higher concentration in the airway epithelium than will the less lipophilic NNK. Such sharp differences in local dosimetry should be considered in order to improve the accuracy of risk assessment models for inhalants. [Research supported by NIH, Grant R01-5910 from NIEHS, and by the Swedish Work Environment Fund, Grant 91-0359 in facilities provided by the U.S. DOE/OHER, Contract No. DE-AC04-76Ev01013.]

237 POLYMERASE CHAIN REACTION AND p53 GENE DNA SEQUENCE IN EXONS 5–9 IN *GAMBUSIA AFFINIS*

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The p53 tumor suppressor gene has been identified in several vertebrate species ranging from man to fish. Mutated forms of the gene have been reported in several tumor types in a variety of species. We have focused on the identification and sequencing of exons 5–9 of the p53 gene in the Western mosquitofish (*Gambusia affinis*). DNA adduct formation and or subsequent mutations in the p53 gene, elicited after exposure to contaminants such as polycyclic aromatic hydrocarbons, may act as biomarkers of potential adverse effects. Fish (3 month old) were acclimatized in the laboratory in fresh, dechlorinated water (pH 8.4, hardness 250 mg/L and 26 °C). Groups of 25 fish (control and exposed) were placed in 20 gallon aquaria with aeration. After exposure to the test compound, fish were euthanitized and the livers removed. Genomic DNA was extracted from the liver tissues using standard procedures. Primers selected from regions between exons 5–8 of the rainbow trout p53 gene sequence were initially used to select for conserved sequences in *Gambusia*. These gene fragments were sequenced and additional primers were selected to isolate a 150 and 600 base pair fragment from the *Gambusia* gene. Direct sequencing of these fragments followed. Mutations occurring in the gene fragments will be determined by PCR-direct sequencing and by single strand chain polymorphism (SSCP).

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238 GENETIC TOXICOLOGY ASSESSMENT OF HI-6 DICHLORIDE

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The oxime HI-6 dichloride [1-(2 hydroxyiminomethyl-1-pyridino)-3-(4-carbamoyl-1-pyridino)-2-oxapropane dichloride monohydrate] has shown to be a potent reactivator of cholinesterase activity and may have efficacy for the treatment of organophosphate intoxication. As part of a preclinical safety assessment program, the genetic toxicology of HI-6 dichloride was evaluated in a series of assays designed to measure induction of gene mutations and chromosomal aberrations. HI-6 gave negative responses in the Salmonella mutagenicity assay and in the CHO/HGPRT gene mutation assay. Dose-dependent increases in the frequency of chromosomal aberrations were noted when HI-6 dichloride was tested in cultured CHO cells and in human peripheral blood lymphocytes. The mouse lymphoma gene mutation assay, reputed to measure both gene mutations and chromosomal deletions, was negative both in the presence and in the absence of metabolic activation. An *in vivo* rat bone marrow metaphase assay was negative. The results from these studies indicate that HI-6 dichloride does not induce gene mutations *in vitro*; however, it is clastogenic *in vitro* but not *in vivo*. (Supported by USAMRMC Contract No. DAMD17-92-C-2001.)

239 INDUCTION OF STABLE LLC-PK1 CLONES WITH DIFFERENT BIO-CHEMICAL CHARACTERISTICS UPON LONG-TERM EXPOSURE TO CARCINOGENS WITH DIFFERENT MECHANISMS OF ACTION

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Long-term exposure of LLC-PK1 cells to low, non cytotoxic doses of the genotoxin dichlorovinylcysteine results in the induction of stable dedifferentiated clones (Vamvakas et al. Toxicol. in press). In the present study we investigated comparatively the characteristics of LLC-PK1 clones induced by 7 weeks exposure to carcinogens with different molecular mechanisms of action: chloroethylcysteine (100 μM), a potent DNA alkylator; cis-platinum (20 nM), a cross-linker; ferric nitrilotriacetate (50 μM), a strand-breaks inducer; potassium bromate (50 μM), an inducer of reactive oxygen species and styrene oxide (5 μM), a potent cytotoxin. Clones established from morphologically altered single cells were maintained in drug-free medium for 4 weeks before investigating their properties. All clones showed decreased cell proliferation, the lowest rates of less than 50 % compared with controls the styrene oxide clone. Membrane polarization assessed by dome formation and sodium-dependent glucose transport, were impaired in all clones compared with the wild-type cells. The impairment was clearly more marked in the styrene oxide clone than in the chloroethylcysteine or cis-platinum clones. The pH-dependent ammonia production, carried out by the mitochondrial glutaminase pathway, was also suppressed in all clones, particularly in the cis-platinum clone, probably due to its potent mitochondrial toxicity. Poly(ADP-ribosy)ation of nuclear proteins induced by DNA strand-breaks was increased more than two-fold in the cis-platinum clone. In contrast, cis-platinum suppressed the expression of the protooncogene *c-fos* compared with the control cells and the other clones. In summary, the general cytotoxin styrene oxide induced clone was mainly characterised by impaired proliferation and membrane transport, while oncogene expression and nuclear protein modification were markedly altered in the clones induced by genotoxic compounds.

240 DETECTION OF ANEUPLOIDY AND BREAKAGE IN THE 1CEN-1Q12 REGION OF HUMAN LYMPHOCYTES INDUCED BY GENOTOXIC AGENTS

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Chromosomal aberrations are associated with cancer, birth defects and pregnancy loss. Previous banding studies on induced chromosomal aberrations have revealed that chromosomal damage induced in human peripheral blood lymphocytes by many genotoxic agents occurs non randomly throughout the genome. One of the regions prone to breakage is the centromeric heterochromatin of chromosome 1. To distinguish hyperdiploidy from breakage occurring in this region, we developed a fluorescence *in situ* hybridization (FISH) procedure using tandem DNA probes. By identifying the number and location of the hybridization signals, interphase nuclei exhibiting breakage affecting the 1cen-1q12 region can readily be distinguished from nuclei hyperdiploid for this region. This hybridization approach was tested by using a series of

known aneuploidy-inducing agents including colchicine, diethylstilbestrol (DES) and vincristine sulfate and the clastogens ionizing radiation, mitomycin C and etoposide. Sodium arsenite and hydroquinone were also tested. Significant increases in alterations were seen with all agents tested and the results were consistent with those previously seen using standard cytogenetic techniques. Treatment with colchicine, DES and vincristine sulfate resulted in high frequencies of primarily hyperdiploidy nuclei whereas cells exposed to radiation, mitomycin C and etoposide exhibited elevated frequencies of breakage. Sodium arsenite and hydroquinone induced relatively minor but significant increases in both hyperdiploidy and breakage. These results indicate that this tandem labeling approach can be used to distinguish aneuploidy-inducing agents from those causing breakage in interphase human cells and may be a valuable procedure for monitoring human populations exposed to genotoxic agents.

241 MUTAGENICITY OF BENZO(A)PYRENE QUINONES: ROLE OF SPECIFIC REDUCTASES

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Benzo(a)pyrene quinones are among the benzo(a)pyrene metabolites that undergo further reductive metabolic activation or detoxification. NADPH: cytochrome P450 reductase (P450 reductase) catalyzed reductive activation of benzo(a)pyrene quinones leading to the formation of semiquinones which enter in the redox cycling and bind specifically to the guanosine residues in the DNA. This binding was prevented by the presence of NAD(P)H: Quinone Oxidoreductase1 (NQO1) activity which competed with the P450 reductase and catalyzed two electron reduction and detoxification of benzo(a)pyrene quinones (P. Joseph and A. K. Jaiswal, PNAS 91: 8413–8417, 1994). In the present study, we have determined the mutagenicity of P450 reductase activated benzo(a)pyrene semiquinones. We used COS1 cell extracts overexpressing cDNA derived P450 reductase to activate the benzo(a)pyrene quinones and *SupF* tRNA as target gene for mutagenicity analysis. We found that binding of P450 reductase activated benzo(a)pyrene semiquinones to the DNA led to mutations well above the level of spontaneous mutations. Many of these mutations involved the base G. Inclusion of cDNA derived NQO1 protein with P450 reductase significantly reduced the frequency of mutations. Increasing the concentration of NQO1 with respect to P450 reductase clearly increased protection to the DNA from benzo(a)pyrene semiquinone induced mutations. These results establish that high expression and induction of NQO1 activity within the cells should provide protection against mutagenicity due to exposure to quinones.

242 α-PARTICLES LIKE THOSE EMITTED BY RADON/RADON PROGENY CAN CAUSE GENETIC CHANGES IN THE ABSENCE OF DIRECT NUCLEAR TRAVERSALS

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We investigated the relationship between nuclear hits by high-LET alpha (α-) particles like those emitted by radon/radon progeny and the subsequent occurrence of sister chromatid exchanges (SCE) in normal human lung fibroblasts (HFL1). Cells were exposed to ^{238}Pu α-particles at doses ranging from 0.4–12.9 cGy and subsequently analyzed for SCE using conventional methods. A significant increase in SCE frequency was observed even at the lowest dose examined, with the extent of induction of SCE in the HFL1 being dose-independent over a dose range of 1.8–12.9 cGy. The percentages of cells that showed excessive SCE following exposure to the α-particles also uniformly increase in a dose-independent manner over this dose range. Based on measurements of the nuclear areas of the HFL1 in conjunction with target theory calculations, the lowest dose resulted in an increase in the percentage of cells showing excessive SCE over the theoretically expected percentage of cells whose nuclei were calculated to be traversed by one or more α-particles. The extent of the discrepancies between theoretically expected and experimentally observed frequencies of SCE became progressively reduced with increasing irradiation dose. These findings are consistent with the existence of an alternative, extranuclear target and mechanism through which α-particles cause DNA damage, as detected by SCE analysis. In a subsequent series of experiments, we have found that the exposure of culture medium only, i.e., no cells present, to α-particles results in the production of a relatively short-lived factor(s) that induces SCE in unexposed cells. We have additionally found that cells also produce a SCE-inducing factor(s) following exposure to α-particles. The existence of an extranuclear compartment as a target for α-particles and an extracellular mechanism(s) for mediating genetic changes may have important implications on the susceptibility of lung cells to the DNA-damaging effects of inhaled radon/radon progeny. This work was funded by the U.S. DOE.

243 CARCINOGEN-INDUCED TRANSLOCATION, GENE CONVERSION AND LOSS OF HETEROZYGOSITY DETECTED IN METABOLICALLY COMPETENT *SACCHAROMYCES* CEREVISIAE STRAINS

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Cancer cells often exhibit genomic destabilization recognized as mutations, chromosomal translocations, gene amplifications, deletions and loss of heterozygosity. Most of these genetic alterations are the result of mitotic recombination events which may occur at elevated levels in cells exposed to carcinogens. To study drug-induced recombination we have constructed a set of metabolically competent *S. cerevisiae* strains that produce human cytochrome P450 enzymes (CYP1A1 or CYP1A2) in combination with human NADPH-cytochrome P450 reductase. The diploid tester strains further carry suitable reporter genes allowing the identification of gene conversion (strain YHE2) or chromosomal translocation (strain YB110) using a simple screen involving direct selection. Our experiments have demonstrated that aflatoxin B1, benzo[a]pyrene-7,8-dihydrodiol and 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole, a class of procarcinogens requiring metabolic activation by CYP enzymes, are efficiently metabolized to products that induce gene conversion as well as translocation.

Mitotic recombination between homologous chromosomes might also contribute to the segregation of heterozygous (tumor suppressor) genes recognized as the phenomenon of loss of heterozygosity (LOH). To address the question whether mitotic recombination is involved in LOH in yeast we have constructed strain YA72 which carries a heterozygous *URA3* gene flanked by additional marker genes. This strain allows us to phenotypically discriminate between crossing-over, gene-conversion, denovo mutation and chromosome loss as the underlying mechanism of *URA3* gene inactivation. Exposure of strain YA72 to various carcinogens produced a dose dependent increase in the frequency of Ura clones and the phenotype of a vast majority of the clones (>95%) was in agreement with mitotic recombination as the responsible genetic mechanism.

244 FIBRONECTIN INHIBITS BLEOMYCIN-INDUCED DNA STRAND BREAKAGE IN MURINE LUNG ENDOTHELIAL CELLS

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Extracellular matrices and integrin receptor activation inhibit apoptosis induced by growth factor withdrawal or cell detachment. Furthermore, matrices and integrins inhibit endotoxin-induced DNA breakage and apoptosis. Bleomycin (**BLM**) causes DNA strand breakage, acute lung endothelial cell injury, and pulmonary fibrosis. Here we tested the effect of the integrin ligand protein, fibronectin (**FN**), on BLM-induced DNA breakage in primary cultures of BALB/c murine lung endothelial cells (**MLEC**). Chambered microscope slides were treated 2 h at 37° with PBS or PBS containing bovine plasma fibronectin (50 μg/ml, 40 μg/cm^2). MLEC were placed in the slides and, after 24 h, were treated for 45 min with 0 to 1 mg BLM/ml. MLEC were fixed immediately after treatment or 2 h after removing BLM. DNA breaks were labelled with fluorescein-12-dUTP by *E. coli* DNA polymerase I. Breaks were measured by nuclear fluorescence intensity in 40-200 cells using a Meridian ACAS 570C laser confocal microscope. BLM caused a concentration-related increase in DNA breaks at 45 min. Breaks decreased during the 2 h washout period, but remained significant. The breaks at 45 min were inhibited by culturing MLEC on FN and returned to basal levels 2 h after removing BLM. Thus in addition to endotoxin, FN inhibits BLM-induced DNA breakage in MLEC. Signaling by integrin extracellular matrix receptors may suppress break formation and/or induce DNA repair. (Supported by PhRMA Research Starter Grant, American Cancer Society Grant IRG-58-34 and an American Heart Association Research Grant).

245 VINYL NORBORNENE (VNB): GENETIC TOXICOLOGY STUDIES

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VNB (CASRN), an industrial chemical which produces hyaline droplet nephropathy in the rat with associated α_{2u} globulin increases, was investigated for *in vitro* and *in vivo* genotoxic potential. A *Salmonella typhimurium* reverse mutation assay (TA98, 100, 1535, 1537 and 1538) was negative without (dose range 0.003–0.3 mg/plate) and with (0.003–0.3 mg/plate) metabolic activation. A forward gene mutation assay in CHO cells (HGPRT locus) showed no significant concentration-related increases in mutation frequencies in the absence (0.01–0.1 mg ml^{-1}) or presence (0.005–0.1 mg ml^{-1}) of metabolic activation. Also, in a sister chromatid exchange (SCE) test in CHO cells, VNB did not cause statistically significant or dose-related increases in the incidence of SCE's in the absence (0.02–0.06 mg ml^{-1}) or presence (0.005–0.03 mg ml^{-1}) of metabolic activation. For a bone marrow chromosome aberration test, groups of 10 male and 10 female Sprague Dawley rats were exposed 6 h/day for 5 consecutive days to (mean ± SD) 0 (control), 48.1 ± 1.29, 146 ± 9.2, or 336 ± 8.5 ppm VNB vapor. Marrow was collected 6 and 24 hr after the final exposure. No statistically significant or exposure concentration-related increases in chromosomal aberrations occurred in VNB exposed animals. With this *in vitro - in vivo* battery of tests, VNB did not show any potential for genetic toxicity.

246 MUTATIONAL SPECTRA IN B6C3F1 *LACI* TRANSGENIC MICE: INSIGHTS INTO *IN VIVO* MUTATIONAL MECHANISMS

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We have used B6C3F1 *lacI* transgenic mice (*lacI* mice) to assess the *in vivo* mutagenicity and mutational spectra of 1,3-butadiene (BD) and ethylene oxide (EO) following inhalation exposures. DNA sequence analysis of *lacI* mutants is used to determine mechanisms of mutagenesis and to calculate the effect of *in vivo* mutant clonal expansion. BD was mutagenic in the bone marrow, resulting in a shift in the spectrum of mutations occurring at A:T base pairs (21%) relative to the background (4%). In the spleen of BD-exposed *lacI* mice, there was also an increased frequency of G:CÆT:A transversions. DNA sequence analysis of *lacI* mutants from BD-exposed mice indicated that *in vivo* clonal expansion of *lacI* mutants ('mutant siblings') is a significant component the *lacI* mutant frequency. Therefore, the *lacI* mutant frequency and mutation frequency cannot be assumed to be equivalent. In EO-exposed mice, an increased *lacI* mutant frequency was observed in 2/3 animals at 200 ppm (6 h/day; 4 weeks). DNA sequence analysis in these animals indicated that the increased *lacI* mutant frequency was due to mutant siblings with G:CÆA:T transitions at CpG sites. These data indicated that the increase observed in EO-exposed *lacI* mice was likely due to the *in vivo* clonal expansion of background *lacI* mutations. The majority of the *lacI* mutants isolated from these studies contained point mutations within the *lacI* sequence, indicating that large deletions are not recovered with this assay. DNA sequence analysis of *lacI* mutants from this mutagenicity assay can provide mechanistic data useful for interpretations of *lacI* mutant frequencies determined after genotoxic exposures.

247 MUTAGENIC EFFECT ON RAT LUNG EPITHELIAL CELLS OF PARTICLES *IN VIVO* AND PARTICLE-ELICITED INFLAMMATORY CELLS *EX VIVO*

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Chronic inhalation of TiO2, carbon black (CB) and quartz (Q) results in lung carcinomas in rats. We have hypothesized that this rat lung tumor response results, at least in part, from the recruitment and activation of inflammatory cells which then exert mutagenic effects on lung epithelial cells. To evaluate this hypothesis we exposed rats by intratracheally instillation to saline or saline suspensions of 10 or 100 mg/kg TiO2, CB or Q particles. Fifteen months after exposure, bronchoalveolar lavage (BAL) was performed and alveolar type II cells were isolated. The alveolar type II cells were cultured in 6 thioguanine (6TG) containing media to select for mutation in the hprt gene. The BAL cells were characterized as to number and type and then co-cultured at a 50:1 ratio with RLE-6TN cells (a rat lung epithelial cell line) for 24 hr. The RLE-6TN cells were then grown in 6TG containing media to select for mutation in the hprt gene. Pulmonary inflammation was observed in all particle exposed rats 15 months after exposure. Hprt mutation frequencies were increased in alveolar type II cells isolated from rats exposed to 100 and 10 mg/kg Q, 100 mg/kg CB and 100 mg/kg TiO2. In addition, an increase in hprt mutant frequency was detected in RLE-6TN cells exposed to BAL cells from rats treated with 100 and 10 mg/kg Q or 100 mg/kg CB. These findings indicate that exposure of rats to inflammatory doses of Q, CB and TiO2 is mutagenic for alveolar type II cells. The demonstration that particle elicited inflammatory cells are mutagenic for epithelial cells *ex vivo* supports an inflammatory cell-dependent mechanism for the *in vivo* mutagenic effects of these particles in rat lung.

248 PHYSICAL CHARACTERIZATION, SOLUBILITY, AND POTENTIAL BIOAVAILABILITY OF ARSENIC IN TAILINGS FROM A FORMER GOLD MINE

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To assess potential human health hazards resulting from exposure to mine tailings with high levels of arsenic (As), ten surface samples were size fractionated, sequentially extracted to dissolve specific minerals, and examined by electron microscopy. Total As concentrations ranged from 115 to 1320 mg/kg; the arithmetic mean concentration was 374 mg/kg. Originally, ore was crushed to facilitate extraction of gold; subsequent weathering of the tailings probably caused additional chemical and physical changes. As a result, more than 80% of the mass of each sample was composed of very fine particles < 250 μm in size. Four samples also contained a large percentage (25–50%) of extremely fine silt and clay (<20 μm). Sequential extraction studies were conducted on a reciprocating shaker for 2 hours at room temperature. Distilled water and 1.0 M magnesium chloride each solubilized less than 1% of total sample As. Extraction with 0.1 M phosphate buffer (pH 8) solubilized 1–14% of total sample As, suggesting that a significant fraction of the As is surface bound and represents a labile pool of soluble As. Final extraction with 0.25 M hydroxylamine hydrochloride in HCl solubilized 4–35% of total sample As, indicating that much of the As is probably associated with surface accumulations of iron which may have developed from oxidation of pyrite. In a separate experiment, when subjected to 0.1 M HCl (pH 2), As dissolution ranged from 24–34%. Examination by scanning electron microscopy revealed that individual particles were composed predominantly of fractured quartz grains and potassium feldspar grains. These observations indicate that as much as 50% of total As in these samples is soluble in aqueous solution and therefore potentially bioavailable.

249 FUTURE RESEARCH PROJECTS THAT COULD IMPACT THE RISK ASSESSMENT FOR ARSENIC IN DRINKING WATER

T D Chinn[1], R L Hund[2]. [1]*HDR Engineering, Inc., Austin, TX;* [2]*American Water Works Association Research Foundation, Denver, CO.* Sponsor: Barbara Beck

The US EPA is considering a new drinking water maximum contaminant level (MCL) between 0.2 and 20 ug/L primarily based on a linear extrapolation of epidemiological studies of exposed Taiwanese populations. Although a relationship between inorganic arsenic in water and human cancer seems likely at high levels, extrapolation to predict potential impact at low levels in the United States has many uncertainties. In order to reduce these uncertainties, an experts workshop was convened in June, 1995, to identify research topics needed to: 1) reduce the uncertainty in the science database used in the risk assessment associated with low level arsenic exposure; and 2) improve the scientific knowledge base on arsenic occurrence and exposure and develop improved and more cost-effective treatment technologies. The 45 workshop participants prepared research project descriptions within the 4 topic areas of Mechanisms of Toxicity; Toxicology/Metabolism/ Modeling; Epidemiology; and Treatment Technologies. A total of 31 research projects were defined with a total estimated funding need of over $19 million. Two of the projects have been funded by the American Water Works Association Research Foundation and additional funding sponsors are being pursued. Timely completion of these research projects will allow the US EPA to perform its arsenic risk assessment with greater certainty. This Poster Session will present the 31 research topics, the estimated time for completion, and their funding status.

250 DEVELOPMENT OF AN IMPROVED METHOD FOR THE DETERMINATION OF ARSENIC AT LOW LEVELS IN BIOLOGICAL MATRICES

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Recent epidemiological studies on the carcinogenicity of arsenic have caused increased concern over the levels of arsenic in drinking water and re-evaluation of the current maximum contaminant level (MCL) of 50 μg/L. The US EPA is considering a new MCL between 0.2 and 20 μg/L primarily based on a linear extrapolation of an epidemiological study in Taiwan. Although a relationship between arsenic and human cancer seems likely at high levels, extrapolation to predict potential impact at low levels in the United States has several inherent uncertainties. Efforts to develop exposure dose-response relations, and the mechanisms(s) of toxic response depend on accurate quantification of arsenic species in large numbers of blood and urine samples. Currently available analytical methods to quantify arsenic species are too time-consuming and expensive to use in studies requiring large numbers of samples. This project will develop or improve current methods to quantify inorganic and arsenic species in blood and urine. In addition, it will develop or improve methods that preserve arsenic in the form it was sampled.

251 ARSENIC INTAKE FROM THE TAIWANESE DIET

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Regulation of environmental arsenic exposures in the United States is based on toxicity values derived from epidemiological studies of a Taiwanese population exposed to arsenic in drinking water. For all populations, the diet also serves as a substantial source of inorganic arsenic exposure. Accurate dose-response assessment for exposed populations should include dietary doses. Insufficient data have previously been available to accurately estimate dietary doses of inorganic arsenic. Preliminary arsenic speciation studies by the Canadian Ministry of the Environment indicated that more than 40 percent of arsenic in rice is inorganic and that inorganic arsenic concentrations in rice (100 ng/g) are higher than those in any other food tested except canned shrimp. Rice and yams were the primary dietary constituents for the exposed Taiwanese population, with smaller amounts of seafood and vegetables. We have measured the inorganic arsenic in rice and yam samples collected in Taiwan and estimate that these foods provided 84 μg of inorganic arsenic per day for a typical person. These data will be supplemented by data from additional recently collected food samples. The relative bioavailability of arsenic in food and water and the implications of these findings for arsenic dose-response assessment will also be addressed.

252 AN ARSENIC DRINKING WATER EXPOSURE PB-PK MODEL PREDICTS ARSENIC STEADY STATE VALUES IN INTERNAL ORGANS

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Arsenic contamination of drinking water occurs throughout the world. Skin and internal organ cancers have been associated with ingestion of arsenic in drinking water in Taiwan. A major uncertainty surrounding the cancer risk estimate from Taiwan is the use of default assumptions by EPA that all ingested arsenic is absorbed and retained internally. Using data from drinking water, gavage, i.p. and i.v. injections and organ, feces and urine analysis in mice, a PB-PK model has been constructed. Arsenate (AsV) is rapidly and almost completely absorbed p.o. AsV is coverted to arsenite (AsIII) and dimethylarsonic acid (DMA) with a transient intermediate of monomethylarsenic acid (MMA). Unlike the default assumption AsV is rapidly excreted. The PB-PK model agrees well with the experimental data. Blood levels of AsIII are not detectable at low exposure suggesting that AsV is converted to AsIII in the tissues. A major conclusion from the PB-PK model is that arsenic burdens rise and fall rapidly with simulated drinking water consumption. The skin burdens of AsIII, as the putative ultimate carcinogen, reach a steady state which is curivilinearly related to the drinking water exposure. At or near the current EPA MCL of 50μg/L the steady state in skin is only slightly affected by the drinking water concentration. These results suggest that the cancer risk estimate for arsenic in drinking water may need revision to incorporate the concept of steady state organ burdens, rather than the current default assumptions. Reduction of arsenic levels in drinking water below the MCL may have little effect on steady state skin arsenic levels.

253 ARSENIC ESSENTIALITY IN LABORATORY ANIMALS

E O Uthus. *USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND.* Sponsor: C O Abernathy

The first strong evidence supporting the essentiality of arsenic for animals became available in the 1970's. Arsenic deprivation caused an increased perinatal mortality in rats, goats, and minipigs and death in some lactating goats, apparently from myocardial abnormalities. Recent studies have suggested that arsenic has a physiological role that affects methyl metabolism through methionine. Consistent effects of arsenic deprivation have been seen in the liver concentration of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH). For example, in female rats fed a diet containing less than 10 ng arsenic/g, the liver concentration of SAM was decreased to 87 ± 6 nmol/g compared to control values of 104 ± 4 nmol/g (mean $\pm$ SE, $p<0.05$). Liver SAH was unchanged by arsenic deprivation and the SAM/SAH ratio was slightly, but not significantly, decreased. Controls received the same diet but supplemented with 0.5 μg arsenic (as As_2O_3)/g. Other research has indicated that alteration of methionine metabolism exacerbates arsenic deprivation. Present studies are designed to ascertain the site of methionine metabolism (transsulfuration or methionine recycling) in which arsenic has its physiological role. The arsenic requirement for growing chicks and rats has been suggested to be around 25 ng/g diet. If arsenic is found to be essential for humans, extrapolation of animal data suggests the requirement of arsenic would be about 12 to 25 μg/day.

254 VALIDATION OF AN ARSENIC MODEL THROUGH URINE AND FECAL MEASUREMENTS

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We have developed a multi-pathway arsenic exposure model. The model uses information on arsenic concentrations in food, water, soil, and dust, combined with estimates of intake and medium-specific absorption. Urinary arsenic is predicted assuming that 60% of absorbed arsenic is excreted in the urine under steady state conditions. Fecal arsenic is predicted assuming all of the unabsorbed arsenic is excreted into the feces. We applied this model at a former copper smelter site. Site-specific distributions were available for the following parameters: soil and dust arsenic concentration (geo. $\overline{X}$ approximately 200 ppm and 100 ppm, respectively); childhood soil and dust ingestion rate (geo. $\overline{X}$ 39 mg/d); soil and dust arsenic bioavailability (geo. $\overline{X}$ 0.20 and 0.28, respectively); exposure duration; water arsenic concentration; air arsenic concentration; and total arsenic levels in food. Monte Carlo simulation was used to predict a daily arsenic uptake and excretion into urine and feces for children. Depending upon the area of the site evaluated, predicted urinary concentrations were from 1.4- to 1.8-fold overpredicted. Fecal arsenic levels were overpredicted by a factor of approximately 5–10. A sensitivity analysis indicated that the most likely reason for overprediction was an overestimate by a factor of about 4-fold of the soil/dust ingestion rate.

255 BACKGROUND CANCER RISKS FROM NATURALLY-OCCURRING ARSENIC

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Inorganic arsenic due to anthropogenic releases at contaminated sites is of concern to public health agencies, and is often an important factor in remediation decisions. Inorganic arsenic can also be naturally-occurring in all environmental media. Average background concentrations of arsenic in food, water, air, and soil in the U.S., as well as daily doses of arsenic to which the U.S. population is exposed are calculated. The total estimated daily oral dose of arsenic for a U.S. resident is approximately 4×10^{-4} mg/(kgxd). This dose, multiplied by the current U.S. Environmental Protection Agency (EPA) oral-administration cancer slope factor (SF), results in a total incremental lifetime cancer risk (ILCR) attributable to background oral sources of 8×10^{-4}, with the majority of arsenic exposure and risk attributable to food and water consumption. The estimated average dose from air inhalation is approximately 8×10^{-6} mg/(kgxd), which results in a total ILCR attributable to background oral sources of 1×10^{-4}. The ILCR range of concern at contaminated sites administered under the U.S. Superfund regulations, representing acceptable de minimus risk, is 1×10^{-6} to 1×10^{-4}. Background sources of arsenic may be as important or more important in terms of exposure and risk when compared to anthropogenic releases, if it is assumed that the EPA-derived arsenic SFs derived are appropriate. However, arsenic SFs are likely to be

a large source of uncertainty, due to extrapolations from high-dose populations with different characteristics from those of the U.S. population. Predicted incidence rates of arsenic-induced skin and internal organ cancers in the U.S., based on the calculated risks, have not been observed in the U.S. population. Quantitative uncertainty analysis of cancer SFs would help resolve these issues.

256 METABOLISM AND TOXICITY OF INORGANIC ARSENIC (AS_i).

D J Thomas[1], M Styblo[2] and C O Abernathy[3]. [1]*NHEERL, US EPA, RTP, NC,* [2]*Univ NC, Chapel Hill, NC,* [3]*Office of Water U S EPA, Washington, DC*

In many species, As_i metabolism involves both redox cycling and oxidative metabolism, yielding mono- and dimethylated As. Reduction in eukaryotes is enzymatic; in prokaryotes, the mechanism is uncertain. Methylation of As_i is catalyzed by methyl-transferases that use S-adenosylmethionine (SAM) as a methyl donor. Internal factors, such as genotype and external ones, e.g., dietary methionine appear to affect reduction and methylation. Inter-human variation in the metabolic pathway leading from As_i to methylated As could profoundly affect As_i exposure. Although a few species (e.g., marmoset) can not methylate As_i, few data exist human methylating variability. Competition between As_i methylation and other pathways that require SAM as a methyl group donor could affect protein and nucleotide methylation as regulatory processes. Future studies should characterize not only the molecular basis of As_i metabolism, but also examine metabolic variation and how it may affect toxicity. (This abstract does not necessarily reflect EPA policy)

257 INTEGRATED ASSESSMENT FOR INGESTED INORGANIC ARSENIC

D W North. *Decision Focus Incorporated, Mountain View, CA.* Sponsor: B D Beck

The purpose of this presentation is to evaluate and quantify the benefits of new research on arsenic in resolving key uncertainties in order to improve risk management decisions regarding arsenic. Ingested inorganic arsenic continues to pose a challenge to toxicologists, epidemiologists, and to regulatory agencies. While the federal decision to promulgate a revised standard for arsenic in drinking water has been deferred, the issues of risk from arsenic exposure remain. The health effects of arsenic at the ambient levels in food and in water remain uncertain. While these effects may not be observable at levels prevailing in the United States, risks projected by EPA's standard methodology to be of the order of 10^{-3} are judged by many to warrant serious concern. Further research might confirm that the cancer risks posed by ambient arsenic in the United States are negligible, that they are at or above the level projected by EPA, or that risks are significant only for a small susceptible subgroup of the population.

For the toxicologist, the challenge is that there is not good animal model for arsenic carcinogenicity. But there are a variety of alternative means to elucidate the mechanisms of action for arsenic carcinogenicity, including investigation of effects in human cell lines in vitro and studies on human populations in developing nations in locations where arsenic in drinking water supplies has led to clear evidence of increased tumor incidence. For the epidemiologist, the challenge is to carry out a new type of quantitative epidemiology, aimed not at establishing whether arsenic is a human carcinogen, but rather at determining the form of the dose-response relationship. This paper concludes that the value of resolving the uncertainty is clearly high, and arsenic deserves high priority for further research.

258 CANCER RISK FROM INORGANIC ARSENIC IN U.S. DRINKING WATER?

K Brown[1] and H Greene[2]. [1]*Kenneth G. Brown Inc. Chapel Hill, NC;* [2]*Atlantic Richfield Company, Los Angeles, CA.* Sponsor: B Beck

Risk estimates of cancer from inorganic arsenic in U.S. drinking water are based on epidemiologic evidence from a region in Southwest Taiwan endemic to Blackfoot disease where arsenic concentrations were as high as several hundred μg/L. Inference of risk for the U.S., where drinking water concentrations are typically less then 5 μg/L but exceed the EPA interim drinking water standard of 50 μg/L in some communities, requires extrapolation from (1) high-dose to low-dose, and (2) across cultures, from Taiwan to the U.S. These extrapolations require assumptions that may be contentious, e.g., low-dose linearity versus a threshold effect resulting from methylation and detoxification at low doses. The current presentation considers other sources of uncertainty resulting from disperse arsenic exposure measurements, data stratification, default model selection for low-dose estimation, and differential consumption of arsenic from food in Taiwan and the U.S. It is concluded that while the Taiwan studies previously used for extrapolation of risk to the U.S. (Tseng, 1968; Chen, 1985) are adequate for hazard identification, they are not well suited to dose-response analysis and, in particular, estimation of risk at low arsenic concentrations in the U.S.

259 ADEQUACY OF THE EPIDEMIOLOGIC DATA BASE FOR RISK ASSESSMENT OF ARSENIC IN SOIL

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Major epidemiologic studies that have investigated exposures to arsenic (As) in water or air (occupational) indicated that both noncarcinogenic and carcinogenic adverse health effects may occur. Information concerning adverse health effects in humans exposed to As in soil and/or indoor dust and non-occupational airborne As are not well documented. Analysis of hair and urine from exposure investigations where exposures occurred from ingestion of soil and/or indoor dust or exposure to airborne particulates containing As indicated that arsenic had been absorbed. Biologic monitoring, however, does not evaluate health outcomes from exposure to As. Based on the information currently available, the adequacy of the existing data base for evaluating threats to human health from exposure to As in soil/dust is limited.

[1]The opinions expressed in this manuscript are those of the authors and do not necessarily reflect the opinions or policies of the Agency for Toxic Substances and Disease Registry or Washington Department of Health.

260 ASSESSING PUBLIC HEALTH THREATS FROM EXPOSURE TO ARSENIC IN THE ENVIRONMENT: ADEQUACY OF METHODS AND THE RELEVANCE TO HEALTH BASED GUIDELINES

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At the majority of the sites in the US reporting Arsenic (As) as a substance of concern that are evaluated by federal, state, or local governments, As is located in soil and/or indoor dust. After the concentration of As in soil or indoor dust has been determined, plausible exposure scenarios for As are formulated; and a dose for the most sensitive segment of the population that is likely to be exposed is estimated. The likelihood that noncancer adverse health effects will be observed is evaluated using benchmarks or guidelines developed for As such as Minimal Risk Levels (MRLs) or Reference Doses (RfDs). Two of the factors affecting the reliability of human health evaluations were found to be (1) assessing exposure to indoor concentrations of As and (2) bioavailability of As in soil/dust (MRLs and RfDs were derived from human exposure to As in water).

[1]The opinions expressed in this manuscript are those of the authors and do not necessarily reflect the opinions or policies of the Agency for Toxic Substances and Disease Registry or U.S. Environmental Protection Agency.

261 POTENTIAL HIGH-END OFFSITE EXPOSURE AND IMPLIED CANCER RISK FOR THE PERMITTED USE OF 1,3-DICHLOROPROPENE IN CALIFORNIA (1995)

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Potential high-end offsite exposure and theoretical lifetime cancer risk have been estimated for the 1995 permitted use of 1,3-dichloropropene (1,3-D) in California. Inhalation exposure estimates were determined for the highest potentially exposed subpopulation, i.e., farm families living in areas of soil fumigant use, through air dispersion modeling and stochastic ("Monte Carlo") exposure simulation. No other exposure pathway is relevant since this subpopulation has no potential for direct dermal contact with the material and the literature indicates no significant potential for oral exposure. Residue studies with radiolabeled *cis- and trans*-1,3-D have shown rapid degradation of the compounds with incorporation of the radiolabel into natural plant constituents and groundwater sampling indicates no routinely detectable 1,3-D. Based on the current analysis, anticipated high-end offsite 1,3-D exposures within California (1995) are on the order of 0.0065 to 0.055 μg/kg/day

(lifetime average daily dose) and corresponding theoretical lifetime individual cancer risks are on the order of 10^{-7} to 10^{-6}.

262 PENTACHLOROPHENOL AND ITS OXIDATIVE METABOLITES INTERFERE WITH MICROTUBULES *IN VITRO*

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To clarify the aneuploidogenic potential of the fungicide pentachlorophenol (PCP) and its oxidative metabolites tetrachlorocatechol (TCC), tetrachloro-1, 2-benzoquinone (TCCQ) and tetrachloro-1, 4-benzoquinone (TCBQ), we have studied the ability of these compounds to interact with microtubule proteins (MTP) under cell-free conditions. All four compounds inhibit the polymerization of microtubules (MT) in a concentration-dependent manner, reaching approximately 50% inhibition at 200 μM PCP and TCC, and 100% at 20 μM TCCQ and TCBQ. The quinones TCCQ and TCBQ bind covalently to MTP; this binding involves cysteine residues of MTP, as the number of free sulfhydryl groups is lower in the adducts than in the control MTP. Moreover, binding to MTP and inhibition of MT assembly by TCCQ and TCBQ is completely prevented by glutathione (GSH). By contrast, the inhibition of MT assembly by PCP and TCC does not involve SH-groups and is not affected by GSH.

In cultured V79 Chinese hamster lung fibroblasts, PCP causes a weak induction of micronuclei containing whole chromosomes as shown with CREST antikinetochor antibodies.

Our results imply that PCP and its metabolites TCC, TCCQ and TCBQ are potential aneuploidogens.

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263 MEMANTINE EFFECTS ON ELECTROGRAPHIC SEIZURES AND SEIZURE RELATED BRAIN DAMAGE RESULTING FROM SOMAN INTOXICATION

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Excitatory amino acids have been implicated in the seizures and seizure related brain damage (SRBD) resulting from soman intoxication. Memantine (Mem) is an open channel blocker of the glutaminergic NMDA receptor subtype which has reported anticonvulsant activity against soman. The aim of these experiments was to evaluate the effect of Mem on electrographic seizure activity (EGSA) and SRBD produced by soman. Male rats were implanted with cortical screw electrodes for recording a bipolar electrocorticogram and allowed to recover for 5–7 days. EGSA was precipitated by injecting soman (180 μg/kg, sc) 30 min after HI6 (125 mg/kg, ip) pretreatment. Saline, Mem(18 mg/kg), atropine (A; 10 mg/kg) or Mem + A was administered i.p. either 5 or 40 min after the onset of EGSA. Saline-treated animals displayed limbic convulsive behaviors and EGSA lasting for 4 or more hours. Mem or Mem + A suppressed the behavioral signs of soman intoxication, but did not terminate EGSA or prevent SRBD. These results indicate that soman-induced seizures are not ameliorated by Mem or Mem + A, and suggest that the presence or absence of soman-induced seizures cannot be reliably verified through behavioral observations alone.

264 INDUCTION OF RAT HEPATIC CYP 2B AND 1A ISOZYMES BY PERINATAL EXPOSURE TO LOW LEVELS OF TECHNICAL GRADE PESTICIDES

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Despite public concern regarding the potential health effects of pesticide exposure during pregnancy and childhood, much less experimental data exists regarding pesticide effects at these life stages than for adult animals. The current study examines the biochemical effects of perinatal exposure to three technical grade pyrethroid insecticides: cypermethrin, bioallethrin and resmethrin and two technical grade dichlorodicarboximide fungicides vinclozolin and iprodione. Groups of N = 9–11 time impregnated female Sprague Dawley rats were injected with technical grade pesticides s.c. at a dose of 25 mg/kg/d active component from gestational d 13–20. At birth pups were sexed and culled to 4 male and 4 female animals/litter and continued to be injected with the same s.c. dose of pesticide from age 1 to 25 d. One pup of each sex/litter was sacrificed at age 21 d, 35 d, 55 d and 85 d. Livers were excised and microsomes prepared from the male pups. Alkoxyresorufin O-dealkylase activities associated with CYP 1A and 2B isozymes were assayed spectrofluorometrically and CYP 1A1/2 and CYP 2B apoprotein levels were assessed by Western immunoblot analysis. In microsomes prepared from pups at birth no ethoxyresorufin O-deethylase (EROD) or benzyloxyresorufin O-debenzylase activity (BROD) could be measured in animals from any treatment group. Pentoxyresorufin O-depentylase (PROD) activity was below detection in control and iprodione treated animals. However, this activity was significantly induced by all three pyrethroids (cypermethrin > bioallethrin > resmethrin) and by vinclozolin treatment ($p < 0.05$). Three apoprotein bands were recognized in d 1 liver microsomes by a rabbit polyclonal antibody directed against rat hepatic CYP 2B1. All three bands were significantly inducible ($p < 0.05$) by vinclozolin and to a lesser and variable extent by the three pyrethroids. In microsomes from 21 d pups a similar pattern of induction was observed in PROD and BROD to that seen for PROD at birth. In addition, EROD was now detectable in all groups and significantly inducible only by vinclozolin-treatment. In microsomes from 35 d pups all activities had returned to basal values. Thus, pyrethroids and vinclozolin are capable of crossing the placental barrier and inducing CYP 2B forms in fetal rat liver. CYP 1A activity was not present at birth but was inducible after 21 days of pup treatment with vinclozolin. Induction was transient and rapidly abolished following the end of treatment. Supported in part by EPA R822778 (T.M.B.).

265 TRICLOPYR: PHARMACOKINETICS AND RENAL CLEARANCE IN MALE BEAGLE DOGS

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Plasma levels and urinary excretion of triclopyr (3, 5, 6-trichloro-2-pyridinyloxyacetic acid) were measured in male dogs given oral doses of 0.5, 5 and 20 mg/kg. Additionally, the renal clearance of intravenously administered ^{14}C-triclopyr was defined in anesthetized dogs. Orally administered triclopyr was rapidly and effectively absorbed. The kinetics of triclopyr were evaluated and were non-linear at 20 mg/kg. For all dose levels, the urinary excretion was essentially complete by 72 hr. However, the fraction of the dose excreted in the urine decreased as the dose increased providing clear evidence that the kinetics/elimination of triclopyr was non-linear at 20 mg/kg. Several non-linear processess appeared to be acting in concert to account for the non-linear kinetics. There is good evidence for a high-affinity low-capacity active-secretory process which becomes saturated at low plasma triclopyr concentrations. Plasma protein-binding of triclopyr ranged from ~94–99%, was non-linear and was fit to a model that required two binding sites which limits its renal clearance. At the higher plasma triclopyr concentrations tubular reabsorption occurs, resulting in a decrease in the overall renal clearance of triclopyr, impacting the observed kinetics following higher doses (> 5 mg/kg). These findings provide support that the effects observed in standard dog toxicity studies (i.e. decreased PSP, slight increase BUN and creatinine) represent physiological competition for excretion and not toxicity. The results of basing a human risk extrapolation on a physiological adaptation is conservative and questionable.

266 DIETARY CHRONIC TOXICITY/ONCOGENICITY OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) IN RATS

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Male and female Fischer 344 rats (50/sex/group) were administered 0, 5, 75 or 150 mg/kg/day 2,4-D via their diets for up to 2 years. A satellite group of animals (10/sex/group) was necropsied after 12 months. Body weights and weight gains of high dose males were decreased 6 and 12%, respectively, while the body weights and weight gains of mid- and high dose females were decreased 11 and 24%, and 19 and 43%, respectively, compared to controls. Various hematology, clinical chemistry, and urinalysis parameters as well as organ weights of mid- and high dose males and females were statistically different from controls. Treatment-related histopathological changes were noted in the eyes of both sexes of high dose rats. Generally slight changes were also noted in the liver, heart and lungs at the same dosage. Lesser effects were noted in the lungs of mid-dose females. There was no treatment-related increases in the incidence of tumors in either sex. The NOEL was determined to be 5 mg/kg/day in both sexes of rats.

267 THE EFFECTS OF PERINATAL/JUVENILE PESTICIDE EXPOSURE ON ADULT CNS, IMMUNE, AND REPRODUCTIVE FUNCTION IN RATS

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In 1988, Congress asked the NAS to evaluate the risk posed to children by pesticides in the food supply. The subsequent NAS report ("Pesticides in the Diets of Infants and Children") identified numerous gaps in our knowledge of the effects of early pesticide exposure on adult immune, reproductive, and central nervous system function. A rodent study was designed to address these issues. Female SD rats are dosed from gd 14 to pnd 7, whereupon the pups are directly dosed and allowed to remain with the dam. Another group of treated dams provides samples on pnd 7 for measures of test compound and major metabolites in maternal blood and milk, and in pup blood. Some males are killed pnd 21 and 35 for Sertoli cell count and neurochemical measures. For the neurotoxicity studies, dosing ends on pnd 21 and the pups (10/sex/dose) are evaluated over a 2-month period in a neurobehavioral battery (FOB and motor activity). Cognitive function is evaluated as adults using a repeated-acquisition and performance operant paradigm. For immunotoxicity studies, rats (12/sex/dose) are dosed through pnd 42; at 8–10 wks of age they are evaluated for antibody response to SRBC, splenic subset analysis, NK activity, and mitogen- and alloantigen-stimulated proliferation responses. For reproductive studies, rats are evaluated at birth for anogenital distance, during dosing for reproductive development, and then for reproductive function as adults. Seven compounds are slated for this evaluation; the first study (methoxychlor) is complete, the second (carbaryl) is on-going.

268 DEVELOPMENT OF A FECAL TESTOSTERONE BIOMARKER IN RODENTS AS A NON-INVASIVE METHOD FOR DETERMINING MALE REPRODUCTIVE HEALTH

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An adverse effect on reproductive health is often one of the organism's first reactions to an environmental stressor. Thus the production and survival of young are frequently used to estimate the effect of exposures on free-ranging populations. More specific evaluations of the reproductive health of free-ranging individuals require capture, stressful restraint and often the removal of individuals from the study population. The present study focuses on the development and validation of a non-radiometric method to determine fecal testosterone levels as a biomarker for adverse effects in free-ranging, individual male rodents. For this development Peromyscus sp. was used as a model. A competitive enzyme immunoassay was adapted to measure fecal testosterone by the following steps: Fecal samples from the rodents were collected, dried, crushed and weighed. Samples were extracted using an organic solvent for 1 hour. Water was added, partitioning the testosterone into the organic phase. The organic solvent was removed via a quick freeze technique and evaporated. The extract was reconstituted in assay buffer and the efficiency determined using samples spiked with tritiated testosterone. Of the solvents tested ethyl ether gave the highest extraction efficiency with a recovery of 85.7% ± 0.9 STD. (n = 5) of the radiolabelled testosterone. Experiments are underway to validate the technique using a laboratory colony of Peromyscus before beginning tests with agricultural chemicals.

269 HEPATIC AND EXTRA-HEPATIC BIOCHEMICAL EFFECTS OF BENOMYL IN MALE SPRAGUE DAWLEY RATS

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The toxic effects of methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate (benomyl) were evaluated in male Sprague-Dawley rats (170–230 g). Benomyl (200 mg/kg in olive oil) was administered via gastric gavage. Control animals received same volume of olive oil. Animals were treated for 18 days and fasted daily for 8–12 hours before treatment. A significant decline in body weight (16% decrease) was observed in animals treated with benomyl as compared to controls. There was also a significant decline in liver total protein in benomyl-treated rats. These changes were not accompanied with any significant alteration in hepatic or extra-hepatic reduced glutatione levels. However, lipid peroxidation in benomyl-treated rats was significantly higher (138.7% increase) than controls ($p<0.001$). Serum gamma glutamyl transferase (GGT) activity was elevated in benomyl-treated rats but was not significantly different from control. Benomyl treatment resulted in a significant elevation (71.5% increase) in GGT activity in the ileum. In addition, superoxide dismutase (SDO) levels were not significantly decreased. These findings clearly indicate that benomyl increases lipid peroxidation. This increased lipid peroxidation and the possible generation of free radicals may explain in part the hepatotoxicity and cytotoxicity induced by benomyl. (Supported by NIH grants GM08111 and RR03020)

270 EFFECTS OF SOLVENTS ON RECOVERY OF SELECTED PESTICIDES FROM AN *IN VITRO* PORCINE SKIN MODEL

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Skin exposure to pesticides are a health concern for agricultural workers. Historically, worker's hand exposure has been evaluated by washing or wiping the hands with a solvent-moistened towelette and determining recoveries. Recent evidence indicates that solvents can facilitate percutaneous absorption by: 1) acting as carriers of pesticides, 2) decreasing the skin barrier properties, and 3) by irritating the skin. The purpose of this study was to evaluate the efficiency of 1-propanol, polyethylene glycol, soap and water, or D-TAM® in removing selected pesticides from skin. Alachlor (Al) and glyphosate (Gl) were selected as test pesticides based on their chemical properties. ^{14}C-labeled pesticide was spiked into formulations (Roundup® for Gl and Lasso® for Al) and applied to isolated porcine skin (2.5μg/cm^2). After a 90 min exposure, the skin was wiped with a solvent-moistened gauze. The gauze was analyzed for radioactivity. Based on recovery of radiolabled pesticide, soap and water was the most efficient at removing Gl in the porcine model system. Recovery from the wipe was 48 ± 6%. Recoveries for other wipe solvents were <40%. For Al, 1-propanol, PEG, and soap and water gave recoveries between 42 and 45% in this model system. Two other pesticides, trifluralin and methyl parathion, have been selected for further investigations based on solubility and other chemical properties.

271 DIFFERENCES IN THE PHARMACOKINETICS AND TOXICITY OF ALACHLOR AND ITS ETHANE SULFONATE METABOLITE

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The ethane sulfonate (ESA) metabolite of alachlor is formed by soil microbes *via* glutathione conjugation and subsequent oxidative metabolism. A series of studies was conducted to determine possible health effects and to compare ESA's toxicity to that of parent alachlor. These investigations included: acute oral and subchronic toxicity, teratology, genotoxicity, ecotoxicology and pharmacokinetic studies. Mechanistic investigations were also conducted to determine the oncogenic potential of ESA relative to alachlor. The oral LD50 in rats was found to be >6000 mg/kg, while the trout LC50 and *daphnia* EC50 values were >104 mg/l. The NOEL for subchronic toxicity was 20 mg/kg/day, although equivocal changes at the next higher dose (182 mg/kg/day) were minimal and not considered biologically relevant. The NOEL for developmental toxicity was ≥1000 mg/kg/day. These results indicate that the acute, subchronic and developmental toxicity of ESA is 7-20 times less than that of alachlor. ESA was not genotoxic in Ames and micronucleus assays. Unlike alachlor, ESA underwent little metabolism, was rapidly excreted, and did not accumulate in tissues. These findings are consistent with ESA's overall low degree of toxicity. ESA did not induce the same pre-neoplastic changes caused by alachlor; thus, there is no basis to expect that ESA would produce tumors as found in alachlor chronic bioassays. In summary, results from various studies show that the toxicity of ESA is substantially lower than that of parent alachlor and support the conclusion that ESA is not of toxicological concern.

272 MIXED INHIBITION OF MOUSE HEPATIC MICROSOMAL 2-HYDROXYLATION OF 17β-ESTRADIOL BY FENITROTHION

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Oxidative desulfuration of phosphorothioate insecticides like fenitrothion (FS) (*O,O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate) by cytochromes P450 (P450s) is thought to release atomic sulfur that can covalently bind to,

and therefore inactivate, P450s. Consequently, the metabolism of endogenous substrates by certain P450s might be altered following exposure to a phosphorothioate. In the present study, administration of FS to mice, at a dose as low as 7 mg/kg, inhibited 2-hydroxylation of 17β-estradiol (E_2) by hepatic microsomes. *In vitro* kinetic analyses indicated that concentrations of FS greater than 5 μM markedly inhibited 2-hydroxylation E_2, thereby preventing accurate determination of appVmax and appkm. FS at a concentration of 5 μM increased the appkm for 2-hydroxylation of E_2, but did not alter the appVmax. However, preincubation of 5 μM FS with hepatic microsomes for 5 min reduced the appVmax for 2-hydroxylation of E_2, as well as the appkm, probably by inactivating certain P450s. These results suggest that FS inhibits 2-hydroxylation of E_2 both competitively and noncompetitively. (Supported by NIEHS Grant ES04335 and NIGMS Grant GM15150).

273 A 28-DAY ORAL STUDY IN RATS TO INVESTIGATE THE EFFECTS OF TOXAPHENE ON THYROID FUNCTION

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This study was designed to investigate the effects of Toxaphene on thyroid function when administered orally, via intubation, to 40 male Sprague-Dawley CD® rats. A dose of 100 mg/kg/day was administered for the first three days. Because two animals died, the dose was lowered to 75 mg/kg/day on Day 4. Control animals (40 males) received the vehicle at the same dose volume as administered to the treated animals. Physical observations, body weights and food consumption were recorded for all animals pretest and weekly during the study. On Study Days 1 (predose), 8, 15 and 29, up to ten males each from the control and treated groups were sacrificed and blood samples were collected for thyroid function assays. Thyroid and pituitary glands were weighed and organ/body weight and organ/brain weight ratios calculated. Complete macroscopic postmortem examinations and histopathological evaluation of the thyroids and pituitary were conducted on all animals. Mean thyroid stimulating hormone (TSH) values of treated males were statistically significantly greater than control values at Days 8, 15 and 29. Thyroid weights of Toxaphene-treated animals were higher than control values at Days 8, 15 and 29. Treatment-related thyroid-follicular-cell hypertrophy and hyperplasia were present among males dosed with Toxaphene. There was a progressive increase in the incidence and/or severity of one or both of these lesions with time, comparing the 15 and 29 day sacrifices with the 8 day sacrifice. The hypertrophy and hyperplasia are typical responses to increased TSH levels in rats and they correlated well with the increase in TSH levels and thyroid weights measured in these rats.

274 A CELL CULTURE MODEL TO STUDY THE EFFECTS OF CONVULSANTS AND PESTICIDES ON THE $GABA_A$ RECEPTOR

J Huang and J E Casida, *Environmental Chemistry and Toxicology Laboratory, University of California, Berkeley, CA.* Sponsor: M T Smith

4′-Ethynyl-4-n-(2,3-$^3H_2$)propylbicycloorthobenzoate (^{3}H-EBOB) is a specific radioligand for the $GABA_A$ receptor noncompetitive blocker site (picrotoxin site). The present study characterizes ^{3}H-EBOB binding properties and insecticide-induced modifications of ^{3}H-EBOB binding to cultured cerebellar granule cells obtained from 7 day-old Sprague-Dawley rats. Cerebellar granule cells exhibit specific ^{3}H-EBOB binding from day 3 in culture, which markedly increases to a peak level at day 7. A single and saturable site is involved with K_d 0.48 nM and B_{max} 219 fmole/mg protein. Neither cerebellar astrocytes maintained in culture for 1–2 weeks nor WSS-1 cells, a cell line stably expressing GABA/benzodiazepine binding sites, exhibit any specific ^{3}H-EBOB binding. Picrotoxinin is a competitive inhibitor *in vitro*. The inhibitory potencies of several polychlorocycloalkane insecticides on ^{3}H-EBOB binding vary from nM to μM ranges and are consistent with their mammalian toxicity. Thus, cerebellar granule cells in primary culture possess single, high affinity ^{3}H-EBOB binding sites associated with the $GABA_A$ receptor complex. These cells can serve as a useful *in vitro* model to study the effects of convulsants and insecticides on the $GABA_A$ receptor (supported by NIH grant ES 00049).

275 ROLE OF METABOLISM IN TESTICULAR TOXICITY OF MOLINATE (ORDRAM) IN MALE RATS

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Molinate is a thiocarbamate herbicide widely used in rice culture. Studies conducted for regulatory purposes have indicated that molinate exposure results in reproductive damage in male rats. The present study investigated the hypothesis that a metabolite of molinate causes testicular toxicity. A single dose acute model of molinate toxicity was established. Molinate was administered to Sprague-Dawley rats (100–400 mg/kg, ip) and testes were evaluated histopathologically at 48hrs, 1 and 3 weeks after administration. Dose-response relationships were observed in testicular histopathological endpoints with vacuolization, atrophy and multinucleated giant cells being seen. The lesion increased markedly with time until, at three weeks, germ cells in the seminiferous tubules were almost completely absent. When molinate sulfoxide was administered, it was a more potent testicular toxicant than the parent compound. HPLC analysis of *in vitro* rat liver microsomal metabolism indicates sulfoxidation as the primary metabolic pathway. Interestingly, human epidemiological studies have shown no adverse effects after molinate exposure. Further analysis will compare rat and human liver microsomal metabolic capabilities and will determine whether metabolic differences can offer an explanation for species differences in susceptibility to molinate's testicular toxicity. (Supported by NIOSH Grant #U07-CCU906162-05 and the California Rice Grower's Association.)

276 DIETARY CHRONIC TOXICITY/ONCOGENICITY OF MICROENCAPSULATED 1,3-DICHLOROPROPENE (1,3-D) IN RATS AND MICE

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Male and female Fischer 344 rats and B6C3F1 mice ingested 0, 2.5, 12.5 or 25, or 0, 2.5, 25 or 50 mg/kg/day microencapsulated 1,3-D, respectively, via their diets for up to 2 years. Microencapsulated racemic 1,3-D (starch-sucrose matrix) was stable in rodent feed and was readily bioavailable upon ingestion. Treatment-related changes in both sexes of rats included dose-related decreased body weights (≤14–16%) and minimal basal cell hyperplasia of the forestomach mucosa (non-progressive) at the mid- and high dosages, and an increase in the normally high incidence of foci of altered hepatocytes at all dosages. Pairwise-analysis revealed a statistical increase in benign liver tumors (adenomas) only in high dose group males, however, the incidence in high dose females and mid-dose males were above historical control values. Treatment-related changes in mice were limited to dose-related decreases in the body weights of both sexes (≤11–14%). No treatment-related increases in tumors were observed in mice. The NOAELs and NOELs in both sexes of rats and mice, respectively, were 2.5 mg/kg/day.

277 COMPARATIVE *IN VITRO* AND *IN VIVO* PROTEIN KINASE C ACTIVATION BY SELECTED PESTICIDES AND TRANSITION METAL SALTS

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Evidence indicates that the polyhalogenated cyclic hydrocarbons TCDD, endrin (EN), chlordane (CL), lindane (LD) and DDT, the organophosphorous pesticides chlorpyrifos (CP) and fenthion (FN), the chloracetamide insecticide alachlor (AL), and transition metal salts including cadmium (Cd) and sodium (Cr) induce oxidative deterioration of lipids and biological macromolecules. No information is available regarding whether these xenobiotics can modulate protein kinase C (PKC) which is a critical event signalling the increase in endothelial permeability and cell proliferation. PKC plays a key role in several cellular functions including the transmembrane transduction of many biochemical signals during metabolism, growth and cell differentiation. Female Sprague-Dawley rats were treated p.o. with two 0.25 LD_{50} doses of the selected pesticides and Cd and Cr at 0 hr and 21 hr, and killed at 24 hr. Liver and brain tissues were removed and the activity of PKC was measured using a nonradioactive assay kit. In a separate series of experiments, cultured PC-12 cells were incubated 24 hr with 50, 100 or 200 nM concentrations of the pesticides, while 0.20, 0.40 or 0.60 concentrations of Cd and Cr were employed. PKC activations were observed in the hepatic and brain cytosol fractions by all xenobiotics. Approximately 1.4 to 2.0-fold and 1.6 to 3.5-fold increases in PKC activity in the hepatic and brain cytosol fractions were observed, respectively. In the hepatic tissues, the greatest increases in activities were observed with TCDD, CP, EN and Cd, while CP and FN exerted the greatest increases in the brain tissues as compared to other xenobiotics. In the *in vitro* experiments, the maximum activation of PKC was observed primarily at 100 nM concentrations of the pesticides. The maximum effects were induced by CP, FN and Cr. The results clearly indicate that pesticides as well as Cd and Cr can modulate a vital component of the cell signalling pathway, namely PKC activity. PKC may be a target of free

radicals and oxidative stress, leading to altered cell proliferation and differentiation.

278 ELECTROCHEMICAL DETECTION OF BIOGENIC AMINES IN THE BRAIN AS A POTENTIAL BIOMARKER FOR THE EFFECTS OF DIELDRIN IN WILDLIFE FROM ROCKY MOUNTAIN ARSENAL

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Dieldrin treatment has previously been shown to alter neurotransmitter levels in the brain of rodents. As part of a wildlife biomonitoring program at Rocky Mountain Arsenal (RMA), where dieldrin represents the most important contaminant of concern for wildlife health, an assay for brain biogenic amines has been adapted for analysis of samples from wildlife species. Norepinephrine, epinephrine and dopamine were measured in brain using reversed-phase liquid chromatography and electrochemical detection. Tissue was homogenized in perchloric acid solution containing 3,4-dihydroxybenzylamine as an internal standard. Following centrifugation, the supernatant was filtered and analyzed on a Microsorb-MV C18 column. The elution solvent was 50 mM sodium phosphate containing 0.2 mM ethylenediaminetetraacetic acid, 1 mM sodium octyl sulfate and 10% methanol (pH 3.5). A glassy carbon electrode operated at 750 mV was used to detect peaks, which were quantified relative to the internal standard. The method provides detection limits of approximately 70, 80 and 140 pmol/g tissue for norepinephrine, epinephrine and dopamine, respectively. This assay will be used for analysis of tissues obtained from wildlife species treated with dieldrin in dosing studies as well as those collected at RMA for assessment of potential neurotoxic effects of site contaminants.

279 EFFECT OF PIPERONYL BUTOXIDE (PBO) ON CELL REPLICATION AND XENOBIOTIC METABOLISM IN RAT LIVER

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We have compared the effects of PBO (a non-genotoxic pesticide synergist) and sodium phenobarbitone (NaPB) on markers of cell replication and xenobiotic metabolism in rat liver. Male F344 rats were fed diets containing O(control), 100, 550, 1050 and 1850 mg/kg/day PBO or 0.05% NaPB (43 mg/kg/day) for periods of 7 and 42 days. Replicative DNA synthesis was studied by implanting 7 day osmotic pumps containing 5-bromo-2'-deoxyuridine during study days 0–7 and 35–42. While NaPB and PBO at the lowest dose level had no effect on body weight gain, the body weights of rats given 550, 1050 and 1850 mg/kg/day PBO for 42 days were reduced to 91.5, 88.5 and 70.0% of control, respectively. Treatment with PBO or NaPB increased relative liver weight and induced a midzonal (PBO) or a centrilobular (NaPB) hypertrophy. High doses of PBO for 42 days also produced individual cell necrosis. Hepatocyte Labelling Index values were increased 1.9, 2.1 and 2.0 fold in rats given 550 and 1050 mg/kg/day PBO or NaPB, respectively, for 7 days and 1.7 fold in rats given 1050 mg/kg/day PBO for 42 days. Treatment with PBO or NaPB induced microsomal cytochrome P-450 content and mixed function oxidase enzyme activities. PBO has been reported to produce liver tumours in the rat at doses of ≥ 1050 mg/kg/day, but not at doses of ≤ 500 mg/kg/day. These results suggest that low doses of PBO produce liver enlargement and induction of xenobiotic metabolism, whereas high doses (where the maximum tolerated dose is exceeded) are associated with hepatotoxicity. (Supported by PBO Task Force II.)

280 DIELDRIN AND LINDANE ACTIVATE NEUTROPHILS BY A MECHANISM THAT INVOLVES CALCIUM-INDEPENDENT PHOSPHOLIPASE A_2

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Polychlorinated biphenyls (PCBs) stimulate rat neutrophils to produce superoxide anion (O_2.) by a mechanism that requires phospholipase A_2 (PLA_2)-medicated release of arachidonic acid (AA). Recent studies suggest that the isoform of PLA_2 that is activated by PCBs is calcium-independent and inhibited by halenol lactone suicide substrate (HELSS), an inhibitor that is selective for calcium-independent PLA_2s. Other polychlorinated compounds, including dieldrin and lindane (hexachlorocyclohexane), have been shown to stimulate O_2. production in neutrophils, but the mechanisms involved in activation are unknown. The current study was undertaken to determine if dieldrin and lindane stimulate O_2. production in rat neutrophils by a mechanism that involves calcium-independent PLA_2. O_2 production and ^{3}H-AA release increased with similar concentration-response relations in neutrophils activated with either dieldrin (1–100 μM) or with lindane (1–100 μM). Significant release of ^{3}H-AA was seen in neutrophils stimulated with dieldrin or lindane in calcium-free medium and in the presence of the intracellular calcium chelator BAPTA-AM, suggesting that these agents stimulate a PLA_2 that does not require calcium for activation. In addition, both O_2. production and ^{3}H-AA release were inhibited in a concentration-dependent manner by HELSS (0.1–100 μM). These data are consistent with the hypothesis that lindane and dieldrin stimulate O_2. production by a mechanism that involves calcium-independent phospholipase A_2. Supported by NIH grant ES04911.

281 ACUTE AND SUBCHRONIC NEUROTOXICITY STUDIES IN RATS WITH BOLERO

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Bolero technical was studied for neurotoxic potential in Sprague-Dawley rats following single oral doses of 0, 100, 500 or 1000 mg/kg. Evaluations included clinical signs, functional observational battery and motor activity over a 14-day period, followed by necropsy and neurohistopathological examination. One of 10 high dose females died. Transient clinical signs, functional changes and reductions in mean ambulatory and total motor activity occurred in the mid and high dose groups. There were no treatment related neuropathological lesions observed. There were no adverse effects at the low dose in either sex. In another study, rats were dosed daily by gavage for 13 weeks at 0, 2, 20 or 100 mg/kg. Clinical signs in the 20 and 100 mg/kg/day groups were minimal and transient. Body weights were reduced in the 20 mg/kg/day group females and the 100 mg/ kg/day group males and females. There were no treatment related functional, motor activity or neuropathological changes. Liver weights were increased in the 20 mg/kg/day group males and kidney weights were increased in both sexes at 100 mg/kg/day. Thus, Bolero was not neurotoxic at levels ≤100 mg/kg/day (highest dose tested), despite non-neurological systemic effects at ≥20 mg/kg/day.

282 EVALUATION OF THE NEUROTOXIC POTENTIAL OF NALED IN RATS

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In acute and subchronic studies with naled (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), Sprague-Dawley rats were given single gavage doses of 0, 25, 100 and 400 mg/kg or daily doses of 0, 0.4, 2 and 10 mg/kg for 13 weeks. Observations included clinical signs, body weights, food consumption, functional observational battery (FOB), locomotor activity, brain weight and dimensions, and neurohisto-pathological evaluation. In the acute study, the animals were observed for 14 days after dosing. Several rats in the 400 mg/kg group died. Surviving high dose males had reduced body weight gain. FOB and motor activity evaluations revealed transient dose-related effects in the 100 and 400 mg/kg groups. At 25 mg/kg, limited signs were seen in females but not in males. One week after dosing, none of the naled treated rats showed evidence of neurotoxicity. In the 13-week study, sporadic and transient tremors were seen among high dose females. There were no other treatment related clinical signs or effects on survival, body weight, food consumption, brain weight or dimensions, or neurohistopathology. No differences were found between treated and control groups in FOB or motor activity evaluations in the 13-week study.

283 DEVELOPMENTAL CHANGES IN A-ESTERASE ACTIVITY TOWARD THE OXONS OF TWO PHOSPHOROTHIONATE INSECTICIDES IN MALE RATS

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Esterases can be classified by their interaction with organophosphorus anti-

cholinesterases such as the active metabolites (oxons) of the phosphorothionate insecticides parathion and methyl parathion. A-esterases, which can hydrolyze these compounds, display the highest activities in the liver and blood, and are dependent on calcium as a cofactor. The developmental changes in A-esterase activity toward paraoxon and methyl paraoxon were investigated in hepatic microsomes of male rats of several ages (1, 3, 12, 21, 33, and 80 days). A-esterase activities toward both oxons (based on milligram microsomal protein) increased with age and were 21-fold and 8.8-fold greater, respectively, at 33 days compared to 1 day. When based on wet weight, the activities toward paraoxon and methyl paraoxon were 39- and 16-fold higher at 33 days than at 1 day. Activities toward both compounds were similar at 80 days compared to 33 days. Activities were of a similar magnitude between both compounds. Low levels of A-esterase-mediated hydrolysis in younger animals, along with low levels of other detoxication mechanisms, may play a role in the greater toxicity levels exhibited by the parent insecticides, parathion and methyl parathion, in juveniles compared to adults. (Supported by NIH RO1 ES04394).

284 AN INVESTIGATION OF DETOXICATION ROUTES FOR PARAOXON IN RAT LIVER AND SERUM

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Paraoxon (PO), the active metabolite of parathion, can be detoxified through a non-catalytic pathway by aliesterases (AliE) and a catalytic pathway by calcium-dependent A-esterases. The detoxication pattern of AliE and A-esterases was investigated *in vitro* in the present study. Fifteen nmoles of PO were incubated with liver homogenate or serum for 5 sec, 3, 5, 15, or 25 min; also 2.5, 5, 7.5, 10, 12.5, 15, or 20 nmoles of PO were incubated with liver homogenate or serum for 15 min. Phenyl saligenin cyclic phosphate (PSCP) and EDTA were used to inhibit AliE and A-esterases, respectively. Concentrations of both PO and *p*-nitrophenol (PNP; the leaving group of PO) were determined by HPLC. When AliE were inhibited, the amount of PNP generated by A-esterases increased with an increase in PO concentration or the time of incubation. When A-esterases were inhibited, the amount of PNP generated with time and PO concentration quickly reached a plateau (i.e., as the amount of PO added or the time of incubation increased, only a slightly greater amount of PNP was generated), indicating an almost total phosphorylation of AliE occurring rapidly in liver or serum. Therefore, less PNP was produced by phosphorylation of AliE than by A-esterase-mediated hydrolysis in high concentrations of PO and/or long periods of time. However, a relatively large amount of PNP was generated at a very early time and there was almost total degradation of PO at low concentrations (which may be physiologically relevant) via AliE phosphorylation. These data suggest that AliE may be a more important mode of PO detoxication than A-esterase activity at low PO concentrations. (Supported by NIH RO1 ES04394).

285 INHIBITION OF BRAIN ACETYLCHOLINESTERASE BY CHLORPYRIFOS AND PARATHION IN β-NAPHTHOFLAVONE TREATED RATS

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The insecticides chlorpyrifos (C = S) and parathion (P = S) are bioactivated to potent anticholinesterases. The level of brain acetylcholinesterase (AChE) as well as hepatic and plasma aliesterase activities were investigated after the iv administration of C = S (14mg/kg) or P = S (1.3mg/kg) to female rats treated with β-naphthoflavone (80mg/kg), a CYP1A1 inducer. Also studied were ethoxyresorufin *O*-deethylase (EROD) and pentoxyresorufin *O*-dealkylase (PROD) activities. C = S and P = S caused about 45% and 25% inhibition, respectively, in brain AChE activity. Inhibition increased to 77% and 53% with BNF exposure in the presence of C = S and P = S, respectively. This suggests that activation of C = S and P = S to the active metabolites was increased, consistent with induced C = S and P = S activation observed in microsomes from BNF-treated rats. EROD and PROD activities were induced by about 60-fold and 11-fold, respectively, after BNF exposure. Hepatic aliesterases were inhibited about 93% and 90% with C = S or P = S, and about 98% and 90% with C = S or P = S, respectively, in the presence of BNF. Plasma aliesterases were inhibited about 92% and 70% with C = S or P = S, and about 97% and 80% with C = S or P = S, respectively, in the presence of BNF. Hepatic aliesterases from BNF-treated rats displayed about a 16% decrease in activity from controls suggesting that there were fewer aliesterases available to protect the target, AChE, from organophosphate inhibition. These results suggest that exposure to BNF can alter the effects of OP insecticides by increasing the amount of active metabolite available to inhibit the target AChE. (Supported by NIH RO1 ES04394).

286 A STUDY ON THE BIOLOGICAL EXPOSURE INDEX OF METHYL PARATHION IN RATS

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Methyl parathion (MeP) is an organophosphate pesticide that readily penetrates skin and inhibits serum cholinesterase (ChE) in mammals. A study was carried out to evaluate the relationship between the urinary excretion of 4-nitrophenol (u-4NP), the in vivo metabolite of MeP, and its inhibition (% inh) of ChE. Wistar rats, 5 per dose group, were housed individually in metabolic cages and gavaged with MeP in Mazola corn oil. Five dose groups per sex, ranging from 0 to 7 mg/kg, were evaluated. Activity of serum ChE was determined both at 24-h before and 24-h after dosing. 24-h urine sample, post dosing, was collected from each rat and analyzed for u-4NP and creatinine (Crn). A background % inh of ChE. was observed for all female, but not male, rats of the 0 mg/kg group. For both sexes, a linear correlation was observed between the excretion of u-4NP and the % inh of ChE. Based on this linear relationship, an u-4NP of 14.5 μg/mL or 21 μg/mg Crn would correspond to ~30% inh. of ChE in male rats and ~30% plus background inh. in female rats. It is therefore suggested that the u-4NP could be used as the biological exposure index for MeP in rats. (Supported by CMRP 461, Chang Gung Med Coll and NSC 83-0421-B182-001Z, Taiwan).

287 DEVELOPMENTAL EXPRESSION OF PARAOXONASE IN MICE

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Paraoxonase is a serum enzyme that is involved in the metabolism of several organophosphorus insecticides. This enzyme is found in many species of mammals and exhibits a substrate-dependent polymorphism in humans. Both human and rabbit paraoxonase genes have been cloned and characterized. The present study demonstrates the cDNA sequence of mouse paraoxonase determined by RT-PCR and RACE procedure. The coding region of the mouse cDNA sequence was 78% identical with human and 74% identical with the rabbit sequence. The inferred mouse protein sequence showed 81% homology with the human sequence and 80% with the rabbit sequence. Fluorescence *in situ* hybridization (FISH) mapped the mouse PON gene to chromosome 6 at band A2, a region conserved with the PON region of chromosome 7 in humans. The earlier observation of very low paraoxonase levels in newborn humans prompted us to examine the developmental-expression of serum paraoxonase in rats and mice. Serum paraoxonase levels increased from birth reaching adult levels at day 25 in rats and day 20 in mice. The serum enzyme activity toward paraoxon increased up to 16 fold in rats, while it increased only 3 fold in mice. Northern hybridization showed that the level of mouse paraoxonase mRNA also increased in the liver from birth to adult. These findings are consistent with the observation of higher sensitivity of newborn animals to organophosphates. (Supported by NIH Grant ES-05194).

288 LETHAL INTERACTIVE EFFECTS OF ACUTE ORAL EXPOSURE TO PYRIDOSTIGMINE BROMIDE, PERMETHRIN, AND DEET IN THE LABORATORY RAT

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This study investigated the lethal interaction of pyridostigmine bromide (PB), permethrin, (perm.), and DEET when given to adult male rats by gavage. The study was separated into two phases. Phase I determined the acute oral lethal dose-response relationship of each compound with the vehicle, propylene glycol. Phase II was divided into two portions. One portion (positive control) was a dose-response study using probit units obtained from phase I (Lethal dose [LD] 16, 30, 50, 70, and 84). Dosage solutions for the second (interaction) portion of phase II contained low levels (calculated LD_{16}, additive LD_{32}) of the two compounds while the concentration of the third compound was varied. Rats were fasted overnight, dosed, and observed for 14 days. A

statistically significant increase in lethality occurred when PB, permethrin, and DEET were given concurrently when compared to additive values. Furthermore, solutions containing PB and permethrin or PB and DEET also caused a statistically significant increase in lethality when compared to expected additive values. This information suggests that lethality in this study was more than an additive effect.

289 HISTOCHEMICAL LOCALIZATION OF NEUROPATHY TARGET ESTERASE IN THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM

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A method has been developed for the histochemical localization of neuropathy target esterase (NTE). Neural tissue from both the central and peripheral nervous system was dissected from 18-day old chicken embryos and frozen in liquid nitrogen-cooled isopropane. The tissue was embedded in Tissue-Tek™ embedding medium and 10 μm sections were cut and placed on gelatinized slides. The sections were air dry for 30 min then fixed for 3 min. in 10% formalin. The sections were pre-treated with 40 μM paraoxon or 50 μM mipafox in a 0.1 M sodium maleate buffer (pH 6.0) for 30 min. at room temperature. The slides were drained and stained in a solution of 100 mM sodium maleate, 100 mM sodium citrate, 30 mM copper sulfate and 5 mM potassium ferricyanide, pH 6.0 (Karnovski and Root's solution), containing 1 mM S-(phenyl) thiopropylthioethanoate (a substrate shown to be hydrolyzed by NTE; Biochim Biophys Acta 1250:171) for 4 hrs at 38 °C. The NTE activity was identified as stain manifest in the presence of paraoxon, but absent in the presence of mipafox. Within the optic lobes and cerebellum, NTE activity was observed as dense discrete regions within neuronal somata. Paraoxon and mipafox resistant activity appeared diffuse throughout the cytoplasm. The most pronounced NTE activity was within the myelinated axons of the cerebellar medulla. Activity within non-myelinated axons was low. Staining within peripheral sciatic nerves was low compared to the CNS tissue. NTE activity appeared most concentrated about the periphery of the axon.

290 KINETIC MECHANISMS OF PURIFIED HUMAN SERUM PARAOXONASE AND HUMAN SERUM ALBUMIN

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It was recently reported (*Drug Metab. Dispos.*22:472, 1994) that human serum paraoxonase (PON) hydrolyzes the organophosphate, paraoxon, by an Ordered Uni Bi kinetic mechanism with an initial-burst reaction. However, we have found that, in the presence of 1.0 mM EDTA, purified human serum PON shows no initial burst of p-nitrophenol or any hydrolytic activity in 50 mM glycine, pH 10.5, buffer. Human serum albumin hydrolyzes paraoxon with an apparent initial-burst reaction. Furthermore, the size of the latter burst is proportional to the amount of albumin added, and the estimates of $appk_2$, $appk_3$, and *appKcat* with albumin are similar to those reported. The pre-steady state kinetics of purified human and rabbit serum PONs were studied using stopped-flow spectrophotometric analysis. p-Nitrophenyl acetate and *p*-nitrophenyl caproate were used as substrates in 50 mM Tris/Cl, pH 8.0, with 1.0 mM $CaCl_2$. At 25 °C and 15 °C, no burst phase was detected. We have found that the purified PONs also hydrolyze several fluorescent esters. For example, 4-methyl-umbelliferyl butyrate was used for pre-steady state studies. Again, no burst reaction was detected by spectrofluorometric analysis. Present evidence supports the conclusion that although hydrolysis of paraoxon by human serum albumin may involve a initial-burst reaction, this is not true for human and rabbit serum PONs. Other models for the hydrolytic mechanism of mammalian PONs must now be considered, e.g., the metal-catalyzed hydrolysis by the bacterial phosphotriesterase. (Supported by NIGMS grant 46979 to BNL)

291 CLONING AND EXPRESSION IN *XENOPUS LAEVIS* OOCYTES OF A cDNA ENCODING A MOUSE LIVER A-ESTERASE

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Organophosphate compounds such as paraoxon [*O,O*-diethyl-*O*-(4-nitrophenyl)phosphate] can be hydrolyzed in mammals by a calcium-dependent enzyme often termed A-esterase. In the present study a rabbit anti-mouse A-esterase polyclonal antibody was used to screen a mouse liver cDNA library in *E. coli* y1019r (Clonetech, Palo Alto, CA). Sizes of cDNA fragments in the 92 positive clones were determined by electrophoresis. Three cDNAs within the appropriate size range, based on the molecular weight of A-esterase, were amplified by PCR, and utilized to isolate their corresponding mRNAs by solution hybridization to total poly $(A)^{+-}$ selected RNA. Injection of $mRNA_{88}$ into *Xenopus laevis* oocytes resulted in expression of the EDTA-sensitive production of *p*-nitrophenol from paraoxon, while injection of $mRNA_{80}$, $mRNA_{76}$, or total mRNA did not. These results suggest that $cDNA_{88}$ encodes for a mouse hepatic A-esterase, while $cDNA_{80}$ and $cDNA_{76}$ do not. (Supported by Grant ES04335 from NIEHS).

292 THE TOXICITY OF CYPERMETHRIN IN ISOLATED RAT HEPATOCYTES

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Cypermethrin is one of the synthetic pyrethroid which is currently gaining popularity as a potent insecticide. The present study was designed to investigate the toxicity of cypermethrin on freshly isolated male and female rat hepatocytes. Hepatocytes were isolated by a collegenase perfusion technique. The hepatocytes were exposed to several concentrations of cypermethrin (0.1-0.8 ug/2 million cells). Cell viability, leakages of aspartate transaminase (AST) and alanine transaminase (ALT) were determined throughout 2 hr incubation period. In male study, 0.8 ug treatment was reduced cell viability significantly after 30 min. of incubation. However, the significant effect of 0.4 ug dose on the viability was observed at 60 min. AST and ALT leakage were significantly increased with 0.8 and 0.4 ug doses as early as 30 and 60 min., respectively. While ALT at 0.2 ug concentration was significantly increased at 2 hr. On the other hand, the female study revealed that the effect of higher doses on cell viability was delayed to 60 min. Also, a significantly reduction after 2 hr incubation with 0.2 ug dose. No gender effect on the leakage of AST was observed. While the leakage of ALT started earlier in female vs male at 0.2 ug concentration. Therefore, cypermethrin has hepatotoxic effect on male and female rat hepatocytes with dose and time dependent.

293 CIMETIDINE-INDUCED CHANGES IN CYTOCHROME P450-DEPENDENT METABOLISM OF PARATHION

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Cytochromes P450 (P450s) oxidatively activate and detoxify phosphorothioate insecticides like parathion (PS) (*O,O* diethyl-*O*-*p*-nitrophenyl phosphorothioate). Therefore exposure to foreign compounds which can inhibit certain P450s could potentially alter the acute toxicities of these insecticides. In the present study administration of the histamine blocker cimetidine (CI), a drug known to inhibit certain P450s, surprisingly potentiated the toxicity of PS in mice, but not its oxygen analog paraoxon. *In vitro* studies with mouse hepatic microsomes suggested that CI increased the P450-dependent activation/ detoxification ratio for metabolism of PS, although total activity was decreased. Incubations with human CYP2B6 expressed in a transfected β-lymphoblastoid cell line (Gentest, Woburn, MA) indicated that CI likely does not alter the spontaneous rearrangement of the putative phosphooxythiran intermediate formed from PS by P450s. These data suggest that CI inhibits certain P450s associated with a lower activation/detoxification ratio for PS metabolism than certain P450s which are not inhibited by CI. This could account, at least in part, for the potentiation of the toxicity of PS by CI. (Supported by Grant ES04335 from NIEHS).

294 COMPARISON OF THE TIME-COURSE OF NEUROTOXIC EFFECTS PRODUCED BY CHLORPYRIFOS IN YOUNG AND ADULT RATS

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A recent National Academy of Sciences document (1993) highlighted the lack of data on age-related differences in pesticide effects. To address this problem we are comparing the effects of selected anticholinesterase pesticides, including chlorpyrifos (CPF), in young (postnatal day 17; PND17) and adult rats. We have found PND17 rats to be approximately five-fold more sensitive to CPF. In this study, we evaluated the time-course of an acute dose of orally-administered CPF in male and female rats on PND 17. Behavioral changes were assessed using an age-appropriate functional observational battery and motor activity; cholinesterase (ChE) activity was measured in brain and peripheral tissues. Rats received either CPF (15 mg/kg) or vehicle (corn oil), were tested, and then tissues were taken at 1, 2, 3.5, 6.5, 24, 72, 168, or 336 hours after dosing (n = 6/sex/treatment/time). Maximal behavioral effects and ChE inhibition occurred at 6.5 hours after dosing; this is somewhat later compared to the time of peak effect in adults (3.5 hours). Partial to full recovery of behavioral changes at 24 to 72 hours was evident in rat pups, which was similar to that observed in adult rats. Blood and brain ChE activity in young rats recovered by 1 week after dosing, whereas brain ChE in adults had not recovered at 2 weeks. Thus compared to CPF-treated adults: (1) young rats show similar behavioral changes in terms of effects and recovery, albeit at a lower dose; (2) the maximal effects are somewhat delayed; (3) ChE inhibition tends to recover quicker in pups, and; (4) there is no difference in CPF effects on these test measures between males and females at this age.

295 PYRETHROID INSECTICIDES AS PHOSPHATASE INHIBITORS

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Pyrethroid insecticide (PYR) inhibition of calcineurin (CN) phosphatase activity was examined using isolated enzyme, brain supernatant (Sup) and cultured IMR-32 human neuroblastoma cells. Non-pyrethroid CN inhibitors cyclosporin A (CyA) and FK506 require immunophilins as cofactors. The hypothesis that PYR inhibit CN directly and immunophilins would not diminish the inhibition was tested. The PYR cis-Cypermethrin (c-Cyp), trans-Cypermethrin, cis-and trans-Permethrin, Deltamethrin (Delt), Fenvalerate (Fen) and S-Bioallethrin were added to CN maximally activated by Ca and calmodulin. c-Cyp, Delt and Fen; were tested for effects on the Ca concentration-dependence of CN. These three, CyA and FK506 were evaluated as phosphatase inhibitors in Sup and IMR-32 cells. Neither the Ca-dependent nor the Okadaic acid-inhibitable phosphatase activity toward a 24 amino acid ^{32}P-phospho-peptide substrate were affected by any of the PYR in any system at concentrations up to 1 μM. CyA and FK506 fully inhibited Ca-dependent phosphatase activity in Sup and IMR-32 cells. Ca concentration-dependence of CN with 0.1 μM calmodulin was unchanged by PYR indicating that Ca binding to calmodulin was not inhibited. EtOH was vehicle for all agents and final EtOH concentration was ≤ 0.2%, the maximum concentration which does not inhibit CN. Use of polypropylene or polyethylene glycol-treated glass tubes in assays did not affect CN activity or inhibition by PYR. These data indicate that the PYR are not effective inhibitors of CN or other phosphatases.

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296 ACUTE TOXICOLOGICAL EFFECTS IN RATS TREATED WITH A MIXTURE OF COMMERCIALLY FORMULATED PRODUCTS CONTAINING METHYL PARATHION AND PERMETHRIN

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The study examined the interactions between a commercial formulation of Methyl Parathion (CF-MP) and a commercially formulated product of Permethrin (CF-PMT) in male rats. The acute toxicity (LD50 values) and brain cholinesterase activity were investigated as toxicological endpoints. Results indicated that CF-MP modified the acute toxicity of CF-PMT. When animals were treated with a mixture, the addition of 380 mg/kg of CF-MP reduced the LD50 of CF-PMT[cl] by only 9.0 %; however, when rats received CF-MP at 464 mg/kg, the LD50 of CF-PMT was reduced by 37% ($p<0.001$). Also the authors found that CF-PMT decreased the CF-MP-induced inhibition of cholinesterase activity by 50 % ($p<0.05$). It was interesting to observe that xylene, which is the most abundant component in the vehicle of both formulations, had no effect on the CF-MP-induced inhibition of the cholinesterease activity. There was no relation between lethality and the inhibition of the brain cholinesterase activity in rats treated with mixtures containing CF-MP + CF-PMT or with either commercially formulated product alone. Considering the increased toxicity observed in rats treated with CF-PMT + CF-MP, it would be advisable to investigate further the interactions between both pesticides.

297 TOXICOLOGICAL IMPLICATIONS OF THE MULTIPLE PARAOXONASES (PONS); A MULTI-GENE FAMILY

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Past studies on mammalian PON genes led to the conclusion that this was not a multi-gene family, but recent findings in our laboratory have demonstrated that man and several other mammals have at least three distinct, but related PON genes. The existence of several PON genes agrees with past observations that mammalian tissues seem to contain several organophosphatases with differing substrate specificities and preferences. DNA covering the amino acid coding regions of the three human genes, PON1, PON2, and PON3 has been sequenced; it is deduced that their protein products share 65 to 75% amino acid identitiy. Interestingly, PON2 and PON3 differ from PON1 in missing three bases coding for the residue 105 in PON1. Birds are very sensitive to paraoxon and they do not express PON1. However, turkey and chicken liver express a PON-like protein resembling PON2, with the same 105 codon deletion. PON2 genes are also present in the dog and mouse, and have about 93% identity with the human PON2. Based on mRNA analyses of mouse tissues, PON2 is widely expressed in liver, pancreas, brain, lung, kidney, small intestine, heart, and placenta. We are currently isolating the PON2 and PON3 proteins, as well as expressing them in mammalian cells in culture to identify and characterize their individual, distinctive organophosphatase and other catalytic properties. (Supported by NIGMS grant 46979 to BNL)

298 SUBSTRATE-DEPENDENT POLYMORPHISM OF HUMAN PARAOXONASE IS REVERSED WITH DIAZINON, SOMAN AND SARIN: PARAOXONASE STATUS OF EXPOSED INDIVIDUALS

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Paraoxonase is an HDL-associated serum enzyme that hydrolyzes the toxic oxons of several organophosphorus insecticides (OPs) and the nerve agents soman and sarin. This enzyme exhibits a substrate-dependent polymorphism in human populations. Previous studies demonstrated that a mutation at amino acid residue 192 is responsible for the paraoxonase polymorphism. One isoform, with arginine at position 192 (R_{192}), hydrolyzes paraoxon with a high turnover number, and the second isoform with glutamine at position 192 (Q_{192}) hydrolyzes paraoxon with a low turnover number. Both isoforms hydrolyze chlorpyrifos-oxon (CPO) and phenylacetate at approximately the same rate. The present study shows that the activity polymorphism of this enzyme is reversed for diazoxon (DZO), soman and sarin hydrolysis. R_{192} homozygotes have lower hydrolytic rates for these OPs, while Q_{192} homozygotes have higher rates of hydrolysis. Heterozygotes have intermediate levels of activities. Within groups of each genotype, there is a wide variability of enzyme levels as seen previously with other substrates. Paraoxonase status was determined for two exposed groups, chlorprifos applicators and subjects showing symptoms of OP toxicity following non-occupational exposure. All of the applicators had moderate to high rates of CPO hydrolysis, while the subjects exhibiting symptoms generally had lower rates of OP hydrolysis. Work is in progress to examine the abilities of each isoform to protect against specific organophosphates. (Supported by NIH Grant ES-05194)

299 THE EFFECTS OF FLAVONE ON THE MOUSE HEPATIC MICROSOMAL BIOTRANSFORMATION OF THE INSECTICIDE PARATHION

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Flavonoids, chemicals produced endogenously by many plants, have been shown to have variable effects on cytochrome P450-dependent activities. Nonhydroxylated flavonoids, like flavone (2-phenyl-4H-1-benzopyran-4-one) often activate, while polyhydroxylated flavonoids often inhibit cytochrome P450-dependent activities. In the present study, preincubation of 1 μM flavone with mouse hepatic microsomes resulted in significantly ($p < 0.05$) increased production of paraoxon (*O,O* diethyl-*O-p*-nitrophenyl phosphate) from 30 μM parathion (*O,O* diethyl-*O-p*-nitrophenyl phosphorothioate) (7.94 ± 0.31 nmol/mg protein/10 min in the presence of 1 μM flavone vs 5.81 ± 1.01 nmol/mg protein/10 min in the absence of flavone). Furthermore, preincubation of 100 μM flavone with mouse hepatic microsomes resulted in significantly ($p < 0.05$) decreased paraoxon production from 30 μM parathion (3.61 ± 0.33 nmol/mg protein/10 min). These data indicate flavone exerts a biphasic effect on cytochrome P450-dependent activation of parathion. These results suggest a need for further studies to assess if flavone can affect the acute toxicity of parathion *in vivo*. (Supported by Grant ES04335 from NIEHS).

300 COMPARISON OF THE TIME-COURSE OF NEUROTOXIC EFFECTS PRODUCED BY CHLORPYRIFOS IN MALE AND FEMALE RATS

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Gender differences in susceptibility to environmental chemicals is a factor that should be considered in health risk assessment. To assess the influence of gender on the effects of selected pesticides, we evaluated the time-course of an acute dose of orally-administered chlorpyrifos (CPF) in adult male and female rats. Behavioral changes were assessed using a functional observational battery and motor activity; cholinesterase (ChE) activity was measured in brain sections and peripheral tissues. Rats received either CPF (80 mg/kg) or vehicle (corn oil), were tested, and then tissues were taken at one of these time points: 1, 2, 3.5, 6.5, 24, 72, 168, or 336 hours after dosing (n = 5/sex/treatment/time). The time-course characteristics of CPF depended partially on the test measure and differed considerably between male and female rats. These differences, however, were not evident between male and female 17-day old rat pups. In the adults, peak behavioral changes generally occurred about 3.5 hours after dosing. The onset of functional changes was quicker in females, but recovery was slower. ChE inhibition in blood and brain followed this pattern as well, indicating that the gender difference could be predicted by the ChE inhibition. For both sexes, however, the ChE inhibition recovered markedly slower (1–2 weeks) than did the clinical effects (24–72 hours). These gender-related differences may be due to both toxicokinetic and pharmacodynamic influences.

301 INHIBITION AND RECOVERY OF RAT FETAL BRAIN ACETYLCHOLINESTERASE ACTIVITY AFTER MATERNAL EXPOSURE TO CHLORPYRIFOS

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Activities of acetylcholinesterases (AChE) and pseudocholinesterase (ChE) were measured in fetuses from Sprague-Dawley rat dams treated with a single oral gavage dose of chlorpyrifos (50 mg/kg). Brain and plasma AChE and ChE levels were measured at time intervals from 1 to 96 hours after dosing in both dams and fetuses. In control animals, fetal brain AChE activity per gram of tissue net weight was about 20% that of the dams. AChE levels in brain one hour after dosing fell to approximately 35% of control in both dams and fetuses and to about 10% of control by 12 hours. Characteristic signs of organophosphate neurotoxicity were observed in the dams. Brain AChE began to recover at 48 hours in the fetuses and at 72 hours in the dams. At 96 hours, brain AChE levels were about 25% of control in the dams while fetal brain AChE had recovered to about 75% of control. Brain ChE levels fell to about 50% of control at 1 hour after dosing and to about 10% of control after 12 hours in both dams and fetuses. Levels returned to about 85% of control in both groups at 96 hours. Plasma ChE fell to about 45% of control one hour after dosing and to about 5% of control at 12-24 hours in both dams and fetuses. ChE activity recovered to 17% of control in the dams and 51% in the fetuses after 96 hours. Since the recovery of inhibited AChE after chlorpyrifos exposure depends on *de novo* synthesis of AChE molecules, these results suggest that fetal brains synthesize AChE at a rate which allows a much more rapid recovery from chlorpyrifos intoxication than adults.

302 STRUCTURE-ACTIVITY RELATIONSHIPS OF ORGANOPHOSPHORUS COMPOUNDS AND ESTERASE SENSITIVITY OR METABOLISM

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Organophosphorus compounds are commonly used on agricultural lands and in households for the control of pests. The acute toxicity levels of these compounds varies with changes in the groups around the phosphorus center. A structure-activity relationship study was performed with respect to target site sensitivity and to several metabolic pathways. Eighteen organophosphorus compounds were studied: the phosphorothionates chlorpyrifos, chlorpyrifos methyl, chlorpyrifos propyl, parathion, methyl parathion, quinalphos, azinphosethyl, coumaphos, diazinon, fenitrothion, ethylfenitrothion, and several model compounds, as well as their respective oxons. The sensitivity to oxon inhibition of the target acetylcholinesterase from brain and of the protective aliesterases from liver, as measured by I_{50}'s, did not correlate well with structure. Only oxons with a heterocyclic ring structure with the N in the 2-position were significantly metabolized by hepatic A-esterases. Of two cytochrome P450-mediated reactions on the phosphorothionates, desulfuration (activation) activities for all but one compound were higher in male than female hepatic microsomes and dearylation (detoxication) activities were also higher in male than female hepatic microsomes. Desulfuration showed a weak correlation to hydrophobicity in males and to molar refractivity in females. It appears that only A-esterase activity can be readily related to structure within the series of compounds investigated. (Supported by NIH ES04394).

303 LASER CYTOMETRIC ANALYSIS OF THE DIFFERENTIAL TOXICITY OF PERMETHRIN TO INSECT AND MAMMALIAN CELL LINES

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Endpoints of cellular injury caused by exposure of insect epithelial cell lines derived from *Anopheles gambiae* and *Aedes albopictus* mosquito larvae and a clonal rat hepatocyte cell line (Clone 9) to the synthetic pyrethroid insecticide, permethrin, were evaluated by laser cytometry. Permethrin acts primarily on nerve cells by modulating voltage activated sodium channels and little is known about other endpoints affected in nonexcitable cells. Kinetic analyses of intracellular glutathione (GSH) and intracellular Ca^{2+} content ($[Ca^{2+}]_i$), reactive oxygen species (ROS) production, mitochondrial (MMP) and plasma membrane potentials (PMP), intracellular pH, and gap junctional intercellular communication (GJIC) were performed. *A. gambiae* and *A. albopictus* cells exhibited IC_{50} values of 15 ng/ml and 300 ng/ml respectively after 72 hr exposure to the toxin. The Clone 9 cells did not show any growth inhibition when treated with permethrin even at the highest concentration used (100 μg/ml). *A. gambiae* cells treated at the IC_{50} concentration for 1 hour at room temperature exhibited changes only in MMP indicating sustained depolarization. However, *A. albopictus* cells treated at the IC_{50} and Clone 9 cells (1 μg/ml) for 1 hr exhibited similar changes in ROS, MMP and GSH levels. These studies identify cellular targets of injury in nonexcitable cells caused by permethrin exposure although insect cells are much more sensitive than mammalian cells.

304 SELECTIVE TOXICITY OF CHLORPYRIFOS TO SEVERAL SPECIES OF FISH DURING AN ENVIRONMENTAL EXPOSURE: AN INVESTIGATION INTO THE BIOCHEMICAL MECHANISMS BEHIND THIS TOXICITY

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Chlorpyrifos is frequently used in termite control. A small pond near a construction site was contaminated with chlorpyrifos in runoff resulting in

the death of several species of fish including largemouth bass, bluegill sunfish, and golden shiners, but not mosquitofish. Chlorpyrifos was confirmed in fish liver by analytical chemistry. Inhibition of acetylcholinesterase (AChE) and aliesterases (AliE) was determined in the tissues of exposed fish sampled prior to death. Brain AChE was inhibited 87/93% in bass, bluegill and shiners but only 73% in mosquitofish. Skeletal muscle AChE was inhibited 91/ 97% in bass, shiners, and mosquitofish but only 77% in bluegill. Liver AChE and AliE were inhibited greater than 90% in bass, shiners, and mosquitofish but in bluegill the activities were inhibited only 86% and 77%, respectively. After 60 days, brain AChE activity in exposed mosquitofish was fully recovered, liver AliE activity was recovered to about 50% of control values, but there was little recovery of skeletal muscle and liver AChE activities. The sensitivity of these enzymes to inhibition by chlorpyrifos-oxon was analyzed *in vitro* by determination of IC50's. Mosquitofish brain and liver AChE and liver AliE exhibited less sensitivity to inhibition than these enzymes from the other species. In contrast, mosquitofish skeletal muscle AChE was more sensitive to inhibition compared to the other species. The lower initial level of inhibition of brain AChE and the enzymes' lower sensitivity to inhibition in mosquitofish compared to other species suggest that the species differences in toxicity exhibited in this exposure resulted from species differences in brain AChE sensitivity to inhibition by chlorpyrifos-oxon.

305 CHARACTERIZATION OF CA^{2+}/CALMODULIN-DEPENDENT ENHANCED PHOSPHORYLATION OF HUMAN MICROTUBULE-ASSOCIATED TAU PROTEIN BY THE BRAIN SUPERNATANTS OF DIISOPROPYL PHOSPHOROFLUORIDATE (DFP)-TREATED HENS

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Several organophosphorus compounds, i.e., tri-o-cresyl phosphate (TOCP), *N, N*-diisopropyl phosphofluoroamidate (mipafox), diisopropyl phosphorofluoridate (DFP) produce delayed neurotoxicity in hens, humans, and other sensitive species. A single dose of DFP (1.7 mg/kg, sc.) produces ataxia followed by paralysis in 7–14 days in hens. Recently, we observed Ca^{2+}/calmodulin-dependent increased phosphorylation of a bacterially expressed human tau protein isoform htau40 by the brain supernatants of DFP-treated hens. The increased phosphorylation was completely inhibited by 3 μM mastoparan and partially by the 100 μM KN-62. The increased phosphorylation of tau protein is consistent with the increased phosphorylation of other cytoskeletal proteins, reported earlier, by the DFP-treated hen brain supernatant. In this investigation, we also phosphorylated htau40 by the bacterially expressed hen Ca^{2+}/calmodulin-dependent protein kinase II and compared it with the tau phosphorylated by the endogenous protein kinases present in the brain supernatants of control and DFP-treated hens. The increased phosphorylation by the DFP-treated hen brain supernatant was also accompanied by increased ^{32}P stoichiometry of htau40 and at least two extra spots, compared to control brain supernatant, on the TLC cellulose plates on phosphopeptides mapping. There was also some difference in phosphopeptides maps of htau40 phosphorylated by control brain supernatant and the expressed fused CaM kinase II. The study shows that increased aberrant phosphorylation of tau protein may be involved in the organophosphorus compound-induced delayed neurotoxicity. (Supported in part by NIEHS grant ESO 5154)

306 THE DIFFERENTIAL EFFECTS OF LEAD (Pb) AND CYTOKINES (IFN-γ AND IL-4) ON THE EXPRESSION OF HLA-DR AND INVARIANT CHAIN (Ii) BY HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC)

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It has been reported that the expression of MHC II can be regulated by Pb, IFN-γ, or IL-4. Both basal and cytokine-induced MHC II and Ii expression is coordinated in most instances. To determine whether Pb can modulate MHC II expression in a manner similar to these cytokines, PBMC from several individuals were exposed in vitro to $PbCl_2$, and analyzed 24 h later. As measured by flow cytometry, 10 and 50 μM $PbCl_2$ increased monocyte surface HLA-DR (MHC II) expression by 1.7-fold and 2.2-fold respectively but surface Ii was not significantly affected. In contrast, 20 u/mL IFN-γ and 20 ng/mL IL-4 increased monocyte surface HLA-DR expression by 2.2-fold and 3.1 fold respectively and surface Ii expression by 1.8-fold and 2.1-fold, respectively. As measured by Western blot, 10 μM $PbCl_2$, 50 μM $PbCl_2$, 20 u/mL IFN-γ, or 20 ng/mL IL-4 increased total PBMC cellular HLA-DR expression by 1.9-fold, 2.1-fold, 3.0-fold, or 5.3-fold, respectively. Total Ii (p33 and p35) expression in PBMC was not affected by Pb while it was increased by IFN-γ and IL-4 1.2-fold and 1.5-fold, respectively. Overall, these results suggest that, in vitro, Pb, IFN-γ, and IL-4 can modulate HLA-DR and Ii expression differentially in human PBMC. Supported by NIH grant ES03179.

307 IMMUNE RESPONSE AND PROTECTION OF RATS AND MICE FROM RICIN AEROSOL EXPOSURE AFTER VACCINATION WITH DEGLYCOSYLATED RICIN A CHAIN

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Ricin is a plant toxin consisting of A and B subunits, each with a MW of approximately 30,000. The A subunit is responsible for the potent protein synthesis inhibitory properties of the molecule, but is non-toxic in the absence of the B chain. The deglycosylation of the native ricin A chain (dgA) produces a molecule with decreased liver retention, increased circulatory half life, and increased immunogenicity. Exposure to aerosolized ricin produces acute lung injury and death. We examined the immune response and survival in rats after three immunizations, and in mice after two or three immunizations. In addition, serum titers in a separate group of 10 immunized rats were monitored for 9 months. Rats (Fisher 344, 6/group) were immunized three times and mice (CD-1, 10/group) were immunized two or three times at 3 week intervals with 10 μg of dgA containing 11 μg of alum adjuvant. Three weeks after the last immunization, animals were challenged with a lethal dose of aerosolized ricin (53–55 mg/min/m^3). Sera taken 24–48 hours before each immunization were examined for ricin-specific IgG antibodies by ELISA and an in vitro neutralization assay. All immunized rats and mice survived the aerosol challenge. No significant antibodies were present in rats after a single immunization. All animals responded after two immunizations. In rats, the geometric mean of ELISA titers was 30,900 ± 7,700 and the neutralization titer was 611 ± 159. After three immunizations, the ELISA and neutralization titers rose to 41,000 ± 12,100 and 1,130 ± 295, respectively. The mouse groups responded immunologically in a similar fashion. Subsequent bleeds at 3 week intervals in the rat 9-month immunization group showed a gradual titer decline over the course of the study. These data suggest that two immunizations with dgA can be sufficient to protect against a lethal ricin aerosol exposure.

308 INTERLABORATORY STUDY OF A MOUSE INTRANASAL METHOD FOR COMPARATIVE IMMUNOGENICITY TESTING OF ENZYMES

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A mouse intranasal (i.n.) dosing method was developed to test the relative immunogenicity of enzymes. In this method, various enzyme doses were administered in a detergent matrix, via intranasal instillation on days 1, 3, and 10, and serum samples collected on day 15. The sera were analyzed for enzyme specific IgG1 antibody by an ELISA. The protease enzyme Alcalase® was tested in each study as a benchmark for comparing the immunogenicity of the test enzyme. The purpose of this investigation was to compare results from independent studies conducted at 2 laboratories. Both labs tested Alcalase vs. 3 test enzymes (an amylase, a lipase, and another protease). An ELISA was optimized for each enzyme and used by both laboratories. The anti-enzyme IgG1 titers were analyzed and ED_{50} values determined. A comparison of ED_{50} values provided a relative potency determination for the test enzymes vs. Alcalase. For lab A the potency values were 0.6X, 0.7X, and 4.8X for the protease, lipase, and amylase, respectively, relative to Alcalase. For lab B the relative values were 0.5X, 0.6X, and 2.1X. Thus, both laboratories were consistent in their relative potency assessments of the enzymes tested. The data were also in line with that obtained from earlier guinea pig intratracheal tests. The three studies with Alcalase also permitted a test of the intralab consistency of the ED_{50} determinations. The labs differed slightly in the values obtained, but were internally consistent from study to study (A: 0.27 ± 0.04 μg/ mouse; B: 0.15 ± 0.02 μg/mouse). These results provide an initial verification of the ease and reproducibility of the i.n. model for evaluating enzyme immunogenicity.

309 ACTINOMYCETES ISOLATED FROM MOLDY HOUSES INDUCE THE EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE AND CAUSE PRODUCTION OF NO, TUMOR NECROSIS FACTOR AND INTERLEUKIN-6 IN MACROPHAGE CELL LINE

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People living in moldy houses suffer from respiratory symptoms which are evidently caused by microorganisms present in these buildings. However, the specific causes and the cellular mechanisms of the symptoms are not yet known. Nitric oxide (NO) is a messenger molecule produced by activated macrophages as a nonspecific immune response directed against invading microorganisms. Tumor necrosis factor (TNF) and Interleukin-6 (IL-6) are important inflammatory mediators which are also liberated by macrophages upon exposure to bacteria or fungi. We tested if any of the microbial strains typical for moldy houses are able to induce production of TNF or IL-6, to activate inducible NO synthase (iNOS) and to cause NO production, assessed by nitrate (uM) in the culture medium, in RAW264.7 macrophage cell line. The macrophages were exposed for 24 hrs to 0, 1×10^5, 5×10^5, 1×10^6, 5×10^6 or 1×10^7 spores/10^6 cells of Cladosporium, Aspergillus, Stachybotrys, Penicillium, Candida albicans, or A4 or A91 Actinomycetes. Dose-dependent NO-production, associated with iNOS expression, was induced by Actinomycetes A91 (up to 17 uM/10^6 cells) or A4 (up to 15 uM/10^6 cells) while all the other tested microbes were without any effect, or caused only a slight increase in nitrite levels compared to the controls (0.7 uM/10^6 cells). Moreover, there was a dose-dependent production of TNF (0 – 230 ng/ml) and IL-6 (0.037 – 437 ng/mg) by all doses of Actinomycetes and TNF-production by the highest dose of Penicillium (0.062 – 0.526 ng/ml)) and Cladosporium (0.036 – 3.181 ng /ml). The cell viability was decreased significantly only by Stachybotrys, but it was not related to NO-, TNF- or IL-6-production. These results suggest that Actinomycetes strongly modify functions of macrophages. Supported by the Academy of Finland.

310 INHIBITION OF HUMAN T CELL ACTIVATION BY p-BENZOQUINONE

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p-Benzoquinone (pBQ) has been shown quantitatively to be the important reactive metabolite of benzene, an environmental toxicant found in cigarette smoke and car exhaust. We investigated the effects of pBQ on human T-cell activation and found that it inhibited two activation events: IL-2 production and agglutination. At low doses (5–15μM) pBQ was immunosuppressive but relatively non-toxic (<10% loss of viability over 24 hours). However, 20μM pBQ was clearly apoptotic (a 1.7 fold increase in oligonucleosomes was observed after a 4 hr treatment) and overtly cytotoxic at 24 hours (as determined by trypan blue exclusion). pBQ (5–15μM) inhibited IL-2 production by 60–90% but had little effect on IL-2Rα expression or blast transformation. pBQ (15μM) did not alter cell surface expression of ICAM-1 or LFA-1 suggesting that its effect on agglutination may be due to post-translational modifications, such as phosphorylation of LFA-1, which significantly increases its affinity for ICAM-1. pBQ (10μM) inhibited IL-2 mRNA expression by 70%, which correlated with the inhibition of the early activation gene c-*fos*. IL-2 production was completely resistant to pBQ added 90 minutes after mitogenic stimulation supporting the inhibition of an early activation event. In conclusion, the target of pBQ is dependent upon its concentration, with low doses inhibiting an early activation event crucial to IL-2 production, intermediate doses affecting agglutination and high doses inducing apoptosis.

311 IMMUNE SYSTEM CHANGES IN NORWEGIAN MALES EXPOSED TO POLYCHLORINATED DIOXINS AND DIOXIN-LIKE COMPOUNDS BY CONSUMPTION OF CRABS FROM A POLLUTED FJORD

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Twenty-five hobby fishermen consuming crabs caught in a fjord in Southern Norway polluted by chloroorganic compounds, and 10 men with no consumption of crabs were studied. Blood levels of a number of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/PCDF) and biphenyls (PCB) were measured, and a set of standard clinical chemistry and hematology analyses were performed. Lymphocyte subsets were analyzed by flow cytometry, and natural killer (NK) cell activity and the response to mitogen stimulation were measured. Finally, we determined anti-tetanus toxin and anti-diphtheria toxin antibody levels before and after booster vaccination. A second set of blood specimens was collected two years after the first. Preliminary statistical analyses indicate a negative correlation between PCDD/PCDF levels and serum creatinine, and a positive correlation between PCB levels and liver enzymes. Red blood cell parameters showed a positive correlation with PCB levels, whereas a negative correlation was found between PCDDs/PCDFs and some white blood cell parameters. A positive correlation was revealed between PCDD/PCDF levels and the response to mitogen. There was a negative correlation between PCDDs/PCDFs and serum IgM levels. No correlation between PCDD/PCDF/PCB levels and the response to vaccination was found in the subgroup of men who had served in the armed forces and therefore with certainty had been vaccinated before.

312 OLTIPRAZ REVERSES BENZO(A)PYRENE SUPPRESSED SECONDARY HUMORAL IMMUNE RESPONSE AND ENHANCES SELECTED LIVER ENZYMES IN FEMALE B6C3F1 MICE

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Oltipraz (OTP), a dithiole thione similar to compounds found in cruciferous vegetables, has been found to be chemoprotective against the carcinogens aflatoxin B_1 and benzo(a)pyrene (B(a)P). The purpose of these studies was to characterize the affect of OTP on B(a)P suppressed humoral immune response and on liver function in female B6C3F1 mice. Doses of 10, 30, 100, 300, and 1000 mg/kg of OTP were administered once per day by gavage for 14 days. ELISA evaluation of the primary IgM response to sheep red blood cell (sRBC) demonstrated a decrease in titer at 300 and 1000 mg/kg. *In vitro* evaluation of the T-dependent antibody response by Mishell-Dutton assay (1nM - 1μM) demonstrated no direct effects. Liver enzyme activities were evaluated in pooled samples from each group. No effect on 4-nitrophenol glucuronosyl transferase (UGT 1.6) activity was observed. Glutathione S-transferase (GST Ya), NADPH-menadione oxidoreductase (NMOR), and UDP glucose dehydrogenase activities were increased. Samples of liver and spleen total RNA were analyzed by Northern blot analysis for UGT 1.6, GST Ya, and NMOR mRNA. UGT 1.6, GST Ya, and NMOR mRNA were increased in liver, but none of the mRNA was detected in spleen. In an effort to block B(a)P immunosupression, OTP (100 mg/kg) was co-administered with dermal B(a)P doses of 5, 20, 40 mg/kg. OTP did not affect B(a)P suppression of primary IgM, but significantly reversed 5 mg/kg B(a)P secondary IgG suppression. Overall, OTP was immunosuppressive at doses above 100 mg/kg as assessed by humoral immune response to sRBC, had no affect *in vitro* on the T-dependent antibody response, caused an increase in the activity and message of selected liver enzymes, and at 100 mg/kg protected against 5 mg/kg B(a)P secondary IgG suppression. Supported in part by NIEHS contract ESO 9522 and NIEHS training grant ESO 7087.

313 OPPOSITE EFFECTS OF 2,2',4,4',5,5'-HEXACHLOROBIPHENYL (PCB153) AND 2,3,7,8-TCDD ON THE ANTIBODY RESPONSE TO SHEEP RED BLOOD CELLS (SRBC) IN MICE

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The effect that co-treatment with PCB153 and 2, 3, 7, 8-TCDD has on the antibody plaque-forming cell (PFC) response to SRBC was determined in female B6C3F1 mice. Groups of 8 mice/group were given a single oral dose of PCB153 alone (0, 3.58, 35.8 or 358 mg/kg), TCDD alone (0, 0.1, 1 or 10 μg/kg), and all possible combinations of these doses in corn oil 7 days prior to immunization with SRBC. Four days after immunization body, spleen, thymus and liver weights, and the PFC response to SRBC were determined. Exposure to TCDD alone resulted in a dose-related suppression of the PFC response, with significant suppression at 1 and 10 μg/kg. In contrast, exposure to PCB153 alone resulted in the enhancement of the PFC response at 358 mg/kg. Combined exposure to 358 mg/kg PCB153 and TCDD resulted in no change (PCB153 + 0.1 μg/kg TCDD) or suppression (PCB153 + 1 or 10 μg/kg TCDD) of the PFC response relative to PCB153 alone. However, the PFC response was enhanced (PCB153 + 0.1 μg/kg TCDD), unaffected (PCB153 + 1 μg/kg TCDD) or suppressed (PCB153 + 10 μg/kg TCDD)

relative to corn oil controls. These results suggest that rather than acting as an Ah receptor antagonist PCB153 acts as a physiological antagonist, since by enhancing the PFC response it raises the "set point" response level such that co-treatment with TCDD at an immunosuppressive dose fails to suppress the PFC response relative to corn oil controls, while clearly suppressing it relative to the PCB153 alone control. (This abstract does not reflect EPA policy.)

314 PROCAINAMIDE-HYDROXYLAMINE PREVENTS IMMUNE TOLERANCE INDUCTION IN T CELL CLONES

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Long-term therapy with procainamide is frequently associated with the appearance of serum autoantibodies and in some cases with a syndrome resembling the autoimmune disease systemic lupus erythematosus. Procainamide can be oxidized by microsomes and activated leukocytes to the reactive metabolite procainamide-hydroxylamine (PAHA). We investigated whether PAHA can interfere with tolerance mechanisms *in vitro*. Tolerance of T cells to self antigens is established by either deleting self-reactive T cells or by making them unresponsive to their cognate antigen (anergy). The T cell clones 12.11 and A.E7 were rendered unresponsive to their cognate antigen by T cell receptor engagement with anti-CD3 antibodies. After overnight incubation with plastic-bound anti-CD3, T cells were challenged with antigen and irradiated syngeneic splenocytes as antigen presenting cells (APC). Responsiveness was determined by measuring IFN-γ levels 24 or 48 hr after antigen addition and by assessing proliferation. Proliferation and IFN-γ levels decreased 8–15 fold in cells treated with anti-CD3 compared to untreated cells. Addition of PAHA during the anti-CD3 treatment caused a dose-dependent recovery of the capacity to proliferate and secrete IFN-γ upon antigen challenge. In 12.11 cells, full responsiveness to the cognate antigen was recovered by adding as little as 5 μM PAHA. These results demonstrate that T cells exposed to pharmacologic levels of PAHA during the anergy induction process can still be activated by their cognate antigen presented on APC. We propose that when maturing T cells in the thymus encounter PAHA during positive selection on self antigen, they retain the potential to respond to this antigen when it is encountered in the periphery. Response of these autoreactive T cells to self antigen presented on APC could help B cells with immunoglobulin receptors for self antigen expand and secrete the autoantibodies characteristic of drug-induced lupus.

315 ACUTE ETHANOL EXPOSURE *IN VIVO* DECREASES I-A EXPRESSION ON SPLENIC B CELLS - GLUCOCORTICOIDS ARE NECESSARY AND SUFFICIENT FOR DECREASED I-A

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Preliminary experiments in this laboratory and published reports from this and other laboratories demonstrate ethanol causes cellular depletion in the spleen. Characterizing this cellular depletion and investigating its mechanism were the goals of these experiments. Splenocytes were studied by flow cytometry. Monoclonal antibodies to cell surface markers were paired as follows: B220/I-A, F480/I-A, CD4/CD8, and IgD/IgM. Vehicle controls were included in each experiment. In a time-course experiment mice were gavaged with ethanol at 7.0 g/kg. The most profound effect was a 44.2% decrease in high level I-A expression on B cells at 12 hours. At this 12 hour time point, a dose-response study was performed using 5.0, 6.0, and 7.0 g/kg ethanol. Loss of I-A from B cells was dose-dependent and significant at all dosages. A glucocorticoid antagonist (RU-486) was given alone and in combination with 6.75 g/kg ethanol; RU-486 blocked the decrease in I-A expression, suggesting that glucocorticoids are necessary for loss of I-A. Corticosterone was given in a manner that produces blood levels and kinetics similar to those observed in mice treated with ethanol at 7.0 g/kg. This caused a loss of I-A on B cells essentially identical to that observed when 7.0 g/kg of ethanol was administered. This indicates glucocorticoids are sufficient to decrease I-A expression. Thus, glucocorticoids are necessary and sufficient for decreased I-A expression in ethanol-treated mice. (Supported by NIH AA9505)

316 IMMUNE ALTERATIONS IN GERIATRIC MICE EXPOSED TO DES AND TCDD

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Aged mice (21 ± 1 mo) were exposed to two potent immunotoxicants, one a therapeutic non-steroidal estrogen (diethylstilbesterol; DES) and one an environmental contaminant (2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDD). Both agents produced pronounced, dose-dependent hypocellularity of the already atrophic geriatric thymus. These compounds additionally produced profound alterations in thymocyte CD4 and CD8 surface antigen expression. DES treatment at 1.5 and 6.0 mg/kg/day for five days decreased percentages of $CD4^+8^+$ (DP) thymocytes to 5.3% and 0.6% of control and increased $CD4^-8^-$ (DN) thymocytes to 340% and 380% of control, respectively. Similar TCDD treatment at 10.0 μg/kg/day decreased DP thymocytes to 28% of control and increased DN thymocytes to 290% of control. Treatment with TCDD also resulted in hypocellularity of the bone marrow, in the presence of unaltered immune cell surface antigen expression. In contrast, DES produced significant alterations in the percentages of cells expressing CD45, Mac-1, and CD45R antigens, without altering total cellularity of the bone marrow. In the spleen, both the percentage and total number of cells expressing Thy 1.2 and CD45R were reduced by DES, but not by TCDD. These immune changes in geriatric mice exposed to TCDD and DES are similar in direction, but in some cases (e.g., thymus) more severe than those reported in either young adult or perinatal models. These data suggest that parameters of immunity in geriatric animals may represent highly sensitive targets of immunotoxic chemical exposure.

317 EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) ON HUMORAL IMMUNE RESPONSES AND IL-1β TRANSCRIPTION IN RATS

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TCDD (1,3,10,30 μg/kg) was administered to female Fisher 344 (F344) and Sprague Dawley (SD) rats followed by i.v. sensitization with sheep red blood cells (4×10^8 cells/ml), a T-cell dependent antigen. The anti-SRBC IgM antibody forming cell (AFC) response was measured to compare the sensitivity of humoral immunity to TCDD treatment within these two rat strains. In addition, total RNA was isolated from liver and spleen to assess potential changes in cytokine transcription, specifically IL-1ß; a gene previously identified to be under the regulation of a dioxin responsive element (DRE) in a human keratinocyte cell line. While there was a dose-dependent augmentation of the anti-sRBC IgM AFC in the F344 strain, the SD strain exhibited a dose-dependent inhibition. Based on the role of IL-1ß in the humoral immune response, quantitative RT-PCR was performed to assess the level of IL-1β mRNA transcription in the spleen and liver from both rat strains following TCDD treatment. In the F344 strain 10 μg/kg TCDD induced a 10-fold increase in IL-1β mRNA in the liver, with no enhancement in the spleen, as compared to the vehicles controls. In SD rats 10 μg/kg TCDD induced a 10-fold increase in IL-1ß mRNA in the spleen and a 100-fold increase in the liver. These results suggest that the difference in sensitivity to inhibition of the AFC response between F344 and SD is not due to the induction of IL-1β. However, our findings are consistent with the premise that IL-1ß is under the control of a DRE and induced by TCDD. (Supported by NIH RO1ES 07255 and Training Grant ES 02520).

318 ROLE OF TUMOR NECROSIS FACTOR-α IN KIDNEY CYTOKINE RESPONSES TO BACTERIAL ENDOTOXIN

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Kidney proinflammatory cytokine responses to endotoxin were studied by using ex vivo and in vitro models of precision cut kidney slice cultures. Female B6C3F1 mice were injected iv with either rabbit anti-mouse TNF-α antisera or preimmune serum 1 hr before endotoxin (LPS 2mg/kg). At 24 hr urinary IL-6 excretion was measured. Kidney slices were incubated in transwell plates and TNF-α, IL-1β, and IL-6 release were measured by ELISA. LPS-primed elevation of urinary IL-6 excretion was suppressed by anti-TNF-α antisera pretreatment. Immunohistochemical staining revealed that IL-6 was expressed in glomerular mesangial cells and suppressed by antisera pre-treatment. Cytokine mRNA expressions, examined by competi-

tive RTPCR, were also decreased by anti-TNF. In addition, monoclonal anti-mouse TNF-α and anti-IL-1β were added to LPS-primed kidney slices and kinetics of proinflammatory cytokine responses were studied. IL-6 from the kidney slices were suppressed by anti-TNF. Feedback inhibition by IL-6 on IL-1 and TNF secretion from the kidney was also examined. TNF induced IL-6 secretion from renal glomerular mesangial cells. Taken together these studies reveal that a cytokine network exists in the kidney.

319 EVALUATION OF D-PENICILLAMINE AND MERCURIC CHLORIDE IN THE BROWN NORWAY RAT MODEL OF TH2 MEDIATED AUTOIMMUNE DISEASE

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Chemical-induced autoimmunity represents an area of immunotoxicology which has not been a major focus of research. The Brown Norway (BN) rat is predisposed to TH2 mediated autoimmune diseases and when exposed to chemicals such as mercuric chloride ($HgCl_2$) develops autoimmune disease similar to human systemic lupus erythematosis (SLE). D-penicillamine (DP) is a drug which has been widely used in the treatment of rheumatoid arthritis. Among its adverse effects are immunologically-mediated autoimmune disorders. The objective of this study was to evaluate $HgCl_2$ and DP in the BN rat as a model for evaluating compounds as potential inducers of TH2 mediated autoimmune disease. Female BN rats were exposed s.c. to $HgCl_2$ (1 mg/kg) 3 times a week for 6 weeks. Elevations in IgE levels indicated the peak response occurred on week 2 of exposure. Dose response (0, 0.01, 0.03, 0.1, 0.3 and 1 mg/kg) studies on week 2 revealed a dose related increase in polyclonal activation of B-cells characterized by elevation of serum titers to IgE, anti-dinitrophenol (DNP), anti-sRBC membranes and increased MHC class II expression on splenocytes. DP treatment (0, 45, 112, 225, and 450 mg/kg) by oral gavage 5 days per week resulted in a peak IgE response on week 4 and dose related increases in IgE and anti-DNP titers. Administration of $HgCl_2$ (1 mg/kg) to animals pretreated for 6 weeks with DP resulted in a dose dependent decrease in $HgCl_2$-induced elevation of serum IgE levels. The BN rat model may prove useful for evaluating compounds for their potential to produce chemical-induced autoimmunity.

320 BREAKING OF TOLERANCE AND MHC-DEPENDENT IMMUNE MODULATION BY AUTOIMMUNOGENIC CHEMICALS

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T cell-dependent B cell stimulation have been implicated in the chemical induction of autoimmunity. This was investigated in more detail by co-injecting defined (auto)antigens with or without the well-known autoimmunogenic chemicals $HgCl_2$ and phenytoin, into the footpad of mice. Next, antigen specific Ig production was determined in the draining popliteal lymph node, to evaluate modulation of the responses by the chemicals. Both $HgCl_2$ and phenytoin stimulated IgG_1 responses to the non-self antigens TNP-ficoll (a T cell-independent type 2 antigen) and TNP-ovalbumin (TNP-OVA, a T cell-dependent antigen). In addition, both chemicals but not complete Freunds adjuvant stimulated the IgG_1 response to OVA in mice orally tolarized for OVA, as well as the response to the natural autoantigen cytochrome-C. Moreover, $HgCl_2$ modulates both primary and secondary responses to TNP-OVA towards a Th2 type response in B 10.s mice (H2-s) that are susceptible for $HgCl_2$-induced autoimmunity. In contrast, the response in resistant congeneic B 10.d2 (H2-d) mice is of an "innocent" Th1-type, as assessed by Igproduction and/or delayed type hypersensitivity reactions. Results indicate that autoimmunogenic chemicals can indeed induce T cell-dependent B cell stimulation, can break tolerance for a non-related (auto)antigen, and can differentially modulate immune responses depending on MHC haplotype. These findings may have major implications in understanding the mechanism of chemically induced autoimmunity.

321 COMPARISON OF SAR MODELS FOR ALLERGIC CONTACT DERMATITIS BASED ON HUMAN AND GUINEA PIG DATA

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A structure-activity relationship (SAR) model was developed for chemicals which elicit allergic contact dermatitis (ACD). Chemicals tested by either human (maximization or patch tests) or guinea pig (maximization, FCAT, Buehler) methods were included in the model. Strict criteria were employed for inclusion of a chemical, such as: purity of the chemical; controls used in animal testing; and greater than three case reports of positive patch testing. A computerized SAR program, Multi-CASE, was utilized to evaluate the fragments (biophores) which were associated with the capability to elicit ACD. The chemicals were then separated into sets by human (n = 913) or guinea pig (n = 170) methods. These sets were evaluated for biophores to determine the extent of structural overlap. Each set of chemicals (i.e., human or guinea pig) was then tested by the counter-model. The findings showed similar biophores identified within each model, i.e., electrophilic moieties, aromatic structures, and nucleophilic moieties with a 75% of overlap of major biophores. In addition, 86.5% of the active chemicals identified by the human methods were recognized by the guinea pig SAR model and 83% of the active chemicals identified by the guinea pig methods were recognized using the human SAR model. These findings indicate that guinea pig tests are a good predictor of human sensitization and the guinea pig data can be combined with human data to develop an SAR model to predict chemical sensitizers. The merged model can then be used to investigate the underlying mechanisms of ACD. Supported by NIEHS ES05651.

322 COCAINE IMMUNOTOXICITY: ROLE OF ENDOCRINE DISRUPTION AND TH1/TH2 PROFILES OF CYTOKINE PRODUCTION

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Previous results in our laboratory have demonstrated an enhanced T-dependent antibody response to SRBC following acute cocaine exposure. The enhancement was correlated with a rise in serum corticosterone following cocaine treatment, and the ability of the administration of exogenous corticosterone to mimic the effect of cocaine was confirmed. Strong evidence for a more causal relationship is the lack of an enhancement in adrenalectomized mice following cocaine administration. The role of concomitant epinephrine release from the adrenal was addressed by adrenal demedullation. Eliminating epinephrine, but not corticosterone release, had no effect on the cocaine-induced enhancement of the antibody response. The cocaine-induced changes in neuroendocrine function most likely occur at the level of the brain as evidenced by the central antagonism of CRF, via α-helical CRF, which prevents the cocaine induced enhancement. Taken together, these findings implicate a primary role for disruption of the HPA axis in the effects of cocaine on immune function. As such, cocaine is an excellent probe to characterize endocrine disruption as a mechanism of immunotoxicity. The T-independent antibody response was not enhanced following exposure to either cocaine or corticosterone, thus suggesting the T cell as a target. Current findings confirm a change in Th1/Th2 cytokine profiles, with an increase in Th2 cytokines IL-4 and IL-10 and no change in Th1 cytokines IL-2 and INF-γ. (Supported by NIH Grant DA-08161 and NIH Training Grant ES-07087).

323 HEXACHLOROBENZENE-INDUCED SKIN AND LUNG LESIONS; A COMPARISON BETWEEN BN, LEWIS AND WISTAR RATS

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Hexachlorobenzene (HCB) is a compound that has been shown to have immunomodulating properties in rats as shown by lymphocyte hyperplasia in spleen and lymph nodes and stimulation of antibody responses. HCB exposure of Lewis rats modulates models of autoimmune disease. Studies have also shown that HCB-treated rats have elevated autoantibody levels. These findings and the association in rats of skin lesions with immune effects and less so with accumulation of porphyrins, make an autoimmune etiology conceivable for HCB-induced skin effects. As chemical induced autoimmune diseases are highly dependent on the genetic make up, we investigated the strain dependency of the immune effects of HCB. Following 4-wk dietary treatment, female BN, Lewis and Wistar strain rats showed a dose-dependent increase of liver, spleen and popliteal lymph node weights and serum IgM against ssDNA, which was most prominent in BN and Lewis rats. In contrast, BN appeared far more susceptible than Lewis rats regarding time of onset, incidence and severity of skin lesions, whereas Wistar rats were hardly susceptible. Microscopically, skin lesions were characterized by hyperplasia

of the epidermis and marked proliferation and activation of vessels in the deep dermis with an inflammatory infiltrate consisting mainly of eosinophilic granulocytes in BN and mononuclear cells in Lewis and Wistar rats. In BN and Lewis rats and less so in Wistar rats, HCB caused a dose-dependent activation of endothelial cells of lung venules. At the ultrastructural level, the endothelial cells were characterized by a marked proliferation of Golgi and RER and resembled high endothelial venules (HEV) in lymph nodes and were therefore called HEV-like. The observation that HCB causes strain-dependent lesions in skin and lung, makes an immune etiology conceivable.

324 INHIBITORY EFFECTS OF PENICILLIN G (PCG) AND LATAMOXEF (LMOX) ON ANAPHYLACTIC REACTIONS INDUCED BY THEIR PROTEIN CONJUGATES IN GUINEA PIGS

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Since anaphylactic reactions are known to be inhibited by the existence of a hapten, it is of interest to examine the inhibitory potential of a drug on anaphylactic reactions in animal models, in relation to prediction of incidence of anaphylactic reactions in clinical use. Cutaneous anaphylactic reactions were provoked by intravenous administration of the corresponding antibiotic-ovalbumin (OVA) conjugate in guinea pigs passively sensitized with antiserum to PCG or LMOX. Inhibition reactions were assessed by co-administration of one of the drugs at doses of 1, 10 and 100 mg/kg with the respective antibiotic-OVA conjugate. LMOX inhibited the reactions from the lowest dose tested, whereas the reactions were not affected by administration of PCG at the highest dose. These results coincide with the previously reported in vitro inhibitory effects of these antibiotics on ELISA for antibiotic-specific antibody analysis[1], and suggest a possible mechanism for the higher incidence of clinical anaphylactic reactions caused by PCG than by a new-generation cephalosporin, LMOX.

1) Katsutani, N. and Shionoya, H. (1993). Int. Arch. Allergy Immunol. 100, 128-134.

325 THE EFFECTS OF TWO MECHANISTICALLY DISTINCT COMPOUNDS ON THE CYTOKINE PRODUCTION BY AN *IN VITRO* T CELL SYSTEM

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The immune system is a potential target for the toxic effects of many environmental and pharmacologic agents. Moreover, the alteration of growth factors and cytokines has been shown to be the target of chemical-induced toxicity. We used antigen-specific helper T (T_H) clones as an *in vitro* model system study the effects of two mechanistically distinct chemicals on cytokine production. 1,2-Dichloroethane (DCE) is widely used as an industrial solvent and insecticidal fumigant. 17β-Estradiol (17β-Es) is a hormone and is also the prototype for a class of estrogen-like chemicals found in the environment. We have examined the effects of DCE and 17β-Es exposure on cytokine production by both T_H1-type and T_H2-type murine cell lines. Cells were activated with antigen and irradiated syngeneic antigen presenting cells in the absence or presence of DCE or 17β-Es, and the release of cytokines into culture supernatants was measured over a 72 h timecourse. DCE (0.1 to 1.0 mM) caused a dose-dependent increase in the production of interferon gamma (IFNγ) by the T_H1 cell line F1.A.2, and 50% increase in both interleukin (IL)-4 and IL-6 by the T_H2 cell line 10.5.17. These effects were also observed when the polyclonal T cell activator anti-CD3 was used to activate T_H clones. Preliminary studies have shown that 17β-Es (10 nM) treatment decreased IFNγ production by F1.A.2 cells, but not by another T_H1 cell line. 17β-Es did not alter IL-6 production but did suppress IL-4 production by two different T_H2 cell lines (F4 and 10.5.17). The results from these studies suggest that both DCE and 17β-Es are capable of modulating T cell cytokine production. Supported by NIH Grant ES00040 (NIK and DJR).

326 THE EFFECTS OF DIETHYLSTILBESTROL (DES) IN THE BROWN NORWAY RAT MODEL OF AUTOIMMUNE DISEASE

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The Brown Norway (BN) rat develops an autoimmune disease that is similar to human systemic lupus erythematosis (SLE) but will do so only after chemical exposure to immunomodulatory agents such as mercuric chloride ($HgCl_2$). Like humans with SLE, the BN rat is predisposed to respond immunologically with TH2 response that is directed towards antibody production rather than cell-mediated immune functions; this may be the mechanism by which chemicals induce autoimmune disease in this model. Since estrogen induces a polyclonal activation of B-cells and is implicated in the development of some autoimmune disorders, it was hypothesized that chemicals with estrogenic activity will exacerbate autoimmune disease in the BN rat. DES, a drug with potent estrogenic activity, was used to test the hypothesis. Initial time course studies showed that female Brown Norway rats gavaged with DES (50 mg/kg/day) had increased serum titers of IgE which peaked at week 4. A dose-response study showed that IgE and anti-DNP IgG titers increased in a dose-dependent manner and peaked at a dose of 50 μg/kg/day. Increases in anti-dsDNA IgG and anti-laminin IgG were also observed. The percentage of spenocytes expressing of MHC II were increased and the IL-2 receptor expression was decreased at all doses. The results show that DES increases serum immunoglobulin titers which may exacerbate the onset of autoimmune disease in this model.

327 CELLULAR IMMUNE MEDIATION OF HALOTHANE INDUCED HEPATOTOXICITY

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Halothane, once the anesthetic of choice for general anesthesia, has declined in use since its association with liver complications with a high potential for lethality. There is ample evidence suggesting halothane hepatitis is an idiosyncratic autoimmune reaction brought about by the formation of neoantigens that have been generated by covalent binding of a halothane biotransformation intermediate. The guinea pig is being developed as a sensitive animal model to investigate an immune-mediated mechanism for halothane hepatitis. Male Hartley guinea pigs were exposed to 1.0% halothane, 40% oxygen for 4 hr. Kupffer cells and lymphocytes were isolated on various days post-exposure. Lymphocyte transformation tests (LTT) using various TFA-antigens as well as Kupffer cells were performed. Using gel electrophoresis and Western blotting techniques, it has been demonstrated that Kupffer cells obtained from halothane treated guinea pigs do carry TFA-adducts as recognized by an anti-TFA antibody. Bands were most prominent by day 2 with the majority diminishing by day seven. No TFA-adducts were detected in any other organ macrophages isolated indicating the immune response to be initiated within the liver by Kupffer cells. The LTT showed a four-fold increase in splenocyte proliferation in response to TFA-guinea pig albumin (GSA). Peak levels of sensitization were observed in splenocytes isolated from guinea pigs 28 days after the animals were exposed to halothane. Stimulation occurred after second and third exposures as well. No significant increase in proliferation could be detected with TFA-lysine or GSA. A ten-fold increase in splenocyte proliferation also occurred in response to Kupffer cells from halothane exposed animals. Autologous splenocytes demonstrated more of a response from treated vs control animals and this could be blocked using anti-MHC II antibody indicating the involvement of MHC II. These results demonstrate Kupffer cells to be the initial cells that present altered proteins in the liver to cells of the immune system. This study provides good evidence that halothane hepatitis is mediated by a cellular immune response. (Sponsored by Procter & Gamble Pharmaceuticals).

328 IMMUNOTOXICITY OF ULTRAVIOLET (UV) B IRRADIATION: CORRELATION WITH INTERLEUKIN 6 (IL-6) PRODUCTION

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Susceptibility to UVB-mediated immunotoxicity is known to differ among strains of mice. In the present study we have examined the association between susceptibility to UVB irradiation-induced inhibition of contact sensitization and the production of IL-6 following topical exposure to a contact

allergen. Two strains of mice, BALB/c and C3H/HeN, have been compared that display, respectively, low and medium/high sensitivity to the immunosuppressive effects of UVB light. Topical exposure of BALB/c and C3H/HeN mice to a skin sensitizing concentration (1%) of the contact allergen oxazolone induced vigorous proliferative responses by draining lymph node cells (LNC) of comparable magnitude. Under these same conditions of exposure very substantial differences in the production by draining LNC of IL-6 (measured by enzyme-linked immunosorbent assay) were observed. Whereas supernatants prepared from cultures of BALB/c LNC contained approximately 10ng/ml IL-6, those from C3H/HeN LNC contained on average 0.3ng/ml. Such differences do not, however, reflect a generalized strain variation with respect to IL-6 expression as in each case treatment with oxazolone induced similar increases in the production of this cytokine in the skin. The data reveal an interesting association between susceptibility to UVB-mediated immunosuppression and the ability of activated LNC to elaborate IL-6.

329 α-NAPHTHYLISOTHIOCYANATE STIMULATES NEUTROPHILS TO RELEASE PROTEASES THAT ARE TOXIC TO HEPATOCYTES *IN VITRO*

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Administration of α-naphthylisothiocyanate (ANIT) to rats causes neutrophil-dependent necrosis of bile duct epithelial and periportal parenchymal cells. Previous studies demonstrated that ANIT can activate neutrophils. We hypothesized that proteolytic enzymes from ANIT-treated neutrophils would damage hepatocytes (HCs) *in vitro*. To test this hypothesis, neutrophils were isolated from Sprague Dawley rats and incubated with ANIT for 18 hours. ANIT (6–50 uM) was not toxic to neutrophils as indicated by lactate dehydrogenase release into culture medium. The conditioned medium from ANIT-treated neutrophils (ANCM) was collected, centrifuged, added to isolated HCs and incubated for 8, 16 or 24 hours. Toxicity of HC cultures was indicated by alanine aminotransferase (ALT) release. Conditioned medium collected from neutrophils exposed to 50 or 25 uM ANIT for 16 to 24 hours caused a pronounced release of ALT from HC cultures. The addition of alpha-1-antitrypsin or trypsin/chymotrypsin inhibitor to ANCM-treated HC cultures afforded protection from toxicity. These data suggest that ANIT stimulates neutrophils to release proteases which cause hepatocellular damage *in vitro*. (Supported by NIH grant ES04139).

330 THE EFFECT OF BE INHALATION ON HUMORAL AND CELL-MEDIATED IMMUNITY IN THE C3H MOUSE

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The purpose of this study was to determine the effect of inhaled Be on humoral and cell-mediated Be-specific immunity in the C3H mouse. Groups of female C3H mice were exposed pernasally to Be metal aerosols under conditions resulting in initial lung burdens of 10, 20 and 40 micrograms Be/lung. Before sacrifice, mice were challenged by intradermal injection of $BeSO_4$ in the footpad to evaluate Be-induced delayed-type hypersensitivity. Mice were sacrificed 7, 60, 120 and 240 days later for evaluation of histopathological changes in lung, lung lymphocyte proliferation in response to Be challenge in vitro, and for quantitation of concentrations of Be-specific IgG in serum. Be inhalation resulted in chronic granulomatous pneumonia with multiple discrete interstitial aggregates of lymphocytes. Bronchiole-associated and perivascular lymphocytes were also increased. The severity of the lesions increased with time and Be lung burden. Immunohistochemical staining of lungs from the high dose mice at 240 days post exposure indicated that the majority of the lymphocytes in the aggregates were B cells. Intradermal challenge of mice with Be did not increase footpad thickness at any time post exposure. Isolated lung lymphocytes proliferated in response to the mitogen, phytohemagglutinin, but not in response to Be. Be-specific IgG was detected in 1/7 high-dose mice by 7 days post exposure and 1/6 medium dose and 4/6 high dose mice at 240 days post exposure. Results suggest that Be inhalation affects humoral rather than cell-mediated immunity in the C3H mouse. [Research supported by the Office of Health and Environmental Research, U.S. Department of Energy, under Contract No. DE-AC04-76EV01013.]

331 SYSTEMIC PRIMING ENHANCES PULMONARY IMMUNITY AGAINST RICIN IN MICE

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Although vaccines have been developed that protect from lethality induced by ricin, we have thus far been unable to induce mucosal immunity against ricin to protect the respiratory tract. In order to better understand the humoral response to ricin vaccine, both systemically and in the respiratory tract, we immunized mice intra muscularly (im), intratracheally (itr), or both 2x with ricin A-chain, challenged them 2 weeks later with a lethal ricin aerosol ($\sim LC_{90}$), and compared anti-ricin IgG and IgA in serum and bronchoalveolar lavage fluid (BALF) before and after ricin aerosol exposure. Mice that were systemically immunized, but not exposed to a ricin aerosol, had 1:10,000 IgG titers, but no detectable IgA in the serum, and had 1:10 IgG titers, and no detectable IgA in BALF. On the other hand, mice that were systemically immunized and exposed to ricin aerosol, had 1:100 IgG titers and 1:100 IgA titers in the serum. BALF from this group of mice contained IgG and IgA titers of 1:100. Mice that were immunized itr had IgG titers of 1:1000, but no IgA in the serum, and had IgG and IgA titers of 1:100 in BALF. Mice immunized only itr did not survive ricin aerosol exposure. Thus, the data presented here suggest that in order to induce pulmonary immunity against ricin, animals must first be systemically immunized and boosted via the respiratory tract.

332 INDUCTION OF PULMONARY ANTI-RICIN IgA BY A RICIN-CHOLERA TOXIN B-CHAIN CONJUGATE

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Some toxins, such as have a direct necrotizing effect when in contact with respiratory mucosal tissue. Although a humoral immune response (IgG) can, and has been, elicited in response to systemic administration of ricin, the humoral response induces a ricin-specific circulating IgG in the serum, but no respiratory IgA is induced to protect the respiratory mucosa. To address this problem, we coupled the known mucosal immunogen, cholera toxin (CT) B subunit, to ricin by a modification of the methods published for covalently linking N-2 fluorenyl succinamic acid to carrier protein. The resulting ricin-cholera mucosal immunogen was purified to a single HPLC peak and was used to immunize mice both systemically and via the respiratory tract for comparison. Both subcutaneously administered ricin-CT conjugate and ricin toxoid (25 μg/mouse, 3x, biweekly) induced anti-ricin IgG in both serum (≥1: 10,000) and bronchoalveolar lavage fluid (BALF) (≥1: 10,000), but no IgA in either serum or BALF. Ricin-CT conjugate and ricin toxoid administered via the respiratory tract (intratracheal instillation) produced anti-ricin specific IgA and IgG response in BALF (≥1: 1000, and IgG titer (≥1: 1000), but no IgA titer in serum. When each group was challenged with a lethal ricin aerosol (~18μg/L), only the systemically immunized mice survived.

333 NITRIC OXIDE PRODUCTION INCREASES IN THE ABSENCE OF ENDOGENOUS GLUCOCORTICOIDS

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The subacute toxic effects of the anticancer drug hydroxyurea (HU) are markedly increased in adrenalectomized (ADX) rats (80–100% lethality after 5 days of oral treatment with 300–800 mg/kg/day), but these animals can be fully protected against these effects by administration of exogenous glucocorticoids (GCs) (*e.g.* dexamethasone 100 gmg/kg/day). Considering the multiplicity of effects exerted by GCs, this protection might be attributed to several mechanisms.

In studies on enhanced HU toxicity in ADX rats, we found that whole-body nitric oxide (NO) is markedly increased after ADX. Plasma levels of L-citrulline, which is co-produced with NO in equimolar amounts as a result of NO-synthase breakdown of L-arginine, were used as an index of circulating NO concentrations in intact (I) and ADX rats. The following results emerged: Mean plasma L-citrulline pm SEM (expressed in pM) (n = 5) after treatment with vehicle (V) was 11.24 pm 0.66 in I rats and 45,900 pm 100 in ADX rats; after HU 10 mg/kg: 12.33 pm 0.32 in I rats and 47,900 pm 420 in ADX rats (the latter, $p < 0.01$ *vs* V treatment); after HU 100 mg/kg: 30.44 pm 1.5 in I rats and 60,770 pm 670 in ADX rats (both, $p < 0.01$ *vs* V treatment); after HU 800 mg/kg: 178 pm 1.1 in I rats and 90,480 pm 500 in ADX rats (both, $p < 0.01$ *vs* V treatment).

Glucocorticoid protection against HU toxicity in ADX rats may depend

in part on down-regulation of NO-synthase activity. However, intramuscular administration of an NO-synthase inhibitor (L-NO-arginine) (0.03–30 mg/kg, twice daily x 5 days) throughout HU treatment (300 mg/ day *per os* x 5 days) failed to provide any significant protection against the latter's toxic effects in these animals, perhaps due to the magnitude of the increase in NO-synthase activity caused by the absence of GCs.
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334 THE DISRUPTION OF LUTEINIZING HORMONE SECRETION BY DIMETHYLDITHIOCARBAMATE (DMDC) AND THE DEVELOPMENT OF TOLERANCE

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The ovulatory surge of luteinizing hormone (LH) depends on GnRH release from the hypothalamus. A primary neurotransmitter involved in GnRH release is norepi-nephrine (NE). DMDC and other dithiocarbamates inhibit NE synthesis. To further investigate this CNS mechanism of DTC, we evaluated the effects of the DMDC on brain NE and LH pulsatile release. We compared single and multiple (21 day) exposures to DMDC in long-term ovariectomized rats. A cardiac catheter was used for serial blood sampling on the day of testing. DMDC suppressed both NE levels and pulsatile LH release after a single dose. Multiple doses were without effect on LH and NE suggesting that the rats became tolerant to the effects of DMDC on the neuroendocrine control of LH. Since a single dose of those dithiocarbamates tested delays the ovulatory surge of LH for 24 h and as a result alters litter size, the possibility that continued treatment will not affect pregnancy outcome should be examined. These data emphasize the importance of considering the effect of acute exposure in the risk assessment process.

335 ESTROGENIC AND ANTIESTROGENIC EFFECTS OF NON-STEROIDAL XENOBIOTICS ON THE HEPATIC EXPRESSION OF THE ESTROGEN-REGULATED mRNA STABILIZING FACTOR

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The hepatic expression of the avian apolipoprotein(apo)II, a component of VLDL is regulated by estrogen. Estrogen-mediated accumulation of apoII mRNA is in part due to its stabilization. Estrogenic stabilization of the mRNA is due to estrogen-induced expression of the estrogen-regulated mRNA stabilization factor (E-RmRNASF) that protects the mRNA from targeted endonucleolytic degradation. To determine whether certain commonly encountered xenobiotics altered the expression of the gene encoding for the above factor by mimicking estrogen, 3 week old roosters were given estrogen, tamoxifen, clomiphene, chlordecone, DDT, araclor, methoxychlor, dieldrin, rotenone, toxaphene, lindane, hexachlorophene or bisphenol-A parenterally. Control birds received only the vehicle. Uniformly radiolabeled capped and polyadenylated apoII mRNA, incubated for an hour in the presence of liver cytosolic extracts from birds who received estrogen, tamoxifen, chlordecone, araclor, or hexachlorophene remained very stable, indicating that these agents were indeed estrogenic and stimulated the expression of E-RmRNASF; but was rapidly degraded in similar extracts from control roosters and those treated with clomiphene, DDT, methoxychlor, dieldrin, rotenone, toxaphene, lindane or bisphenol-A. To determine whether the latter agents were anti-estrogenic, roosters were given a 1:5 (wt) combination of estrogen and each of the xenobiotic. ApoII mRNA underwent degradation in liver cytosolic extracts from roosters who received clomiphene, toxaphene or bisphenol-A, indicating that these agents prevented estrogen from stimulating expression of the above gene and were antiestrogenic. The mRNA remained stable in the rest of the extracts indicating that DDT, methoxychlor, dieldrin, rotenone, and lindane did not interfere with estrogenic stimulation of the E-RmR-NASF expression.

336 ENDOCRINE EFFECTS OF PACLITAXEL *IN VITRO*

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Paclitaxel (PCT), a crude extract from the bark of the Pacific yew, *Taxus brevifolia*, belongs to a novel class of antineoplastic agents, the taxoids. Encouraging clinical trials have led to its approval in many countries for the treatment of recurrent ovarian and breast cancer. PCT enhances microtubule assembly and inhibits the depolymerisation of tubulin, which can lead to bundles of microtubules in the cell. The cell becomes blocked during the G_2 and M phases of the cell cycle and cannot form a normal mitotic spindle and divide.

We have studied the effects of PCT on adrenocorticotrophic hormone (ACTH) and prolactin (PRL) release *in vitro*, since several mitotic spindle poisons, such as vincristine and vindesine, have been found able to affect endocrine secretions, with possible implications on toxicity and therapeutic effects (*Brunetti et al., Toxicol. Lett. 75: 69-73; 1995*).

Male rat anterior pituitary cells were dissociated by trypsin, plated into 24 - well dishes (100,000 pituitary cells/dish) and incubated in air/CO_2 95/5% for 2–3 days. Then, they were incubated with graded concentrations (10^{-10} - 10^{-5}M) of PCT for 1 hour.

PCT did not modify ACTH release, while it decreased PRL secretion significantly vs. controls (controls: 3.78 ± 0.20; PCT 10^{-10}M:3.52 ± 0.08; 10^{-9}M: 3.42 ± 0.12*; 10^{-8}M: 3.17 ± 0.09*; 10^{-7}M: 2.14 ± 0.16**; 10^{-6}M: 1.93 ± 0.03**; 10^{-5}M: 1.83 ± 0.12**;PRL ng/ml; *$p<0.05$ vs. controls; **$p<0.01$ vs. controls).

This unique feature could be relevant in therapeutic effect of PCT, as PRL inhibition could influence breast cancer cell proliferation, an effect which could add to the direct antimitotic action of PCT.

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337 EFFECTS OF THE PEROXISOME PROLIFERATOR WYETH 14,643 (WY) IN THE LACTATING GOAT (*COPRA HIRCUS*)

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Some peroxisome proliferators are reported to reduce body weight gain in suckling rats, possibly through a lactational effect to alter milk composition. Wyeth 14,643 (WY) is a hypolipidemic agent and a potent inducer of hepatic peroxisome proliferation in rats and mice, but only a weak inducer in guinea-pigs, non-human primates, and humans. Goats are an excellent model for studying lactation, however their sensitivity to the peroxisome proliferator and plasma lipid-lowering properties of WY has not been evaluated. The present study was conducted to address this, and to determine the effect of WY on milk composition. Six lactating adult female goats were randomly assigned to control and 40 mg/kg WY groups. WY or vehicle was orally administered for 14 consecutive days, and the goats were milked twice daily. WY treatment had no significant effects on final body and liver weights (absolute and relative), milk volume, or its content of total fat, total protein, and total carbohydrate. Serum glucose, cholesterol, and triglyceride levels were lower in the treated group, but only reached statistical significance for glucose ($p \leq 0.05$). Hepatic β-oxidation (control 16.2 ± 0.3, WY 48.1 ± 5.1 nmol/min/mg) and aromatase activities (control 21.7 ± 5.1, WY 31.6 ± 1.2 pmol/min/mg) were significantly greater in the treatment group than in the control ($p \leq 0.05$), but WY did not affect the total content of hepatic cytochrome P450. There was no difference between the aromatase activities in pooled samples of ovarian microsomes. These data suggest that goats are weak responders to the hepatic peroxisome proliferator effects of WY, and that the weak hypolipidemic effect is not acutely accompanied by a lipid-lowering effect on milk.

338 EFFECT OF ZILEUTON AND TESTOSTERONE SUPPLEMENTATION ON THE DEVELOPMENT OF LEYDIG CELL PROLIFERATIVE CHANGE IN AGED FISCHER RATS

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Zileuton, a benzothiophene N-hydroxyurea, is a 5-lipoxygenase inhibitor that increased the incidence of benign Leydig cell tumors in a two-year carcinogenicity study in Sprague-Dawley rats. A non-genotoxic mechanism of tumorigenesis was suggested by lack of clear evidence of genotoxicity. To explore this possibility, zileuton was administered in the diet at dosages of 0 (control) or 170 mg/kg/day to male Fischer 344 rats (9 months old) with or without supplemental testosterone provided as a subcutaneous implant for a period of 24 weeks. Mean serum testosterone levels in testosterone-supplemented groups (control and zileuton) were approximately two-fold higher than levels in unsupplemented groups (control and zileuton) during the course of the study. As would be expected, elevated levels of serum testosterone in the supplemented groups were accompanied by suppression of serum LH levels. Testes were collected and evaluated following 24 weeks of treatment. Consistent with the serum hormone level profile, small testes and testicular atrophy were observed in supplemented groups, whereas minimal to

moderate Leydig cell hyperplasia was observed in unsupplemented groups. Leydig cell tumors were present only in the zileuton-treated, unsupplemented group. Thus, supplementation with exogenous testosterone, resulting in elevated serum testosterone and suppressed LH levels, prevented the development of Leydig cell tumors in zileuton-treated rats. These results suggest an underlying hormonal, epigenetic mechanism for the observed increased incidence of Leydig cell tumors by zileuton.

339 ATRAZINE DISRUPTS HYPOTHALAMIC CONTROL OF PITUITARY-OVARIAN FUNCTION

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We have evaluated the brain-pituitary-ovarian axis of female Long-Evans hooded and Sprague Dawley rats following exposure to the chlorotriazine herbicide atrazine (ATR). In ovariectomized (OVX) rats, ATR (200 mg/kg, i.p. for 3 days) completely suppressed the estrogen-induced surge of luteinizing hormone (LH) and prolactin (prl). However, the pituitaries of these animals did release LH in response to gonadotropin-releasing hormone (GnRH). In hypophysectomized females bearing ectopic pituitary autotransplants, the resultant hypersecretion of prl was not altered by ATR (300 mg/kg, i.p.). In long-term OVX rats, LH is released in a pulsatile fashion, reflecting CNS (GnRH) modulation of this hormone. Using this model, we identified a dose- and time-dependent disruption of pulsatile LH release. In rats exposed to 0, 75, 150 and 300 mg/kg ATR hypothalamic dopamine and norepinephrine concentrations were not affected by either single or continued dosing suggesting that a depletion of CNS catecholamines was not responsible for altered pituitary hormone secretion. Thus, ATR disrupts the CNS control of pituitary function, but the precise mechanism remains elusive.

340 STIMULATION BY PCBs OF INSULIN RELEASE FROM RINm5F CELLS

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Reports indicating possible altered glucose homeostasis resulting from PCB exposure prompted studies of insulin release from clonal insulin producing cells. A comparison of media insulin at various times after application of 10 μg/ml Aroclor 1254, or separately the congeners 3,4,3'4'-TCB, 2,4,2'4'-TCB, or 2,4,5,2'4'5'-HCB indicated that that the Aroclor mixture and the non-coplanar congeners produced insulin release whereas co-planar 3,4,3'4'-TCB was relatively inactive. The maximum effect of active compounds, a 3 to 4 fold increase over control in media insulin occurred during the first hour of exposure. The rate of insulin release declined over the next three hours with no evidence of cell death. The response was concentration dependent and reversible. Insulin release with Arocolor 1254 was similar to that produced by depolarization of cells with 20 mM KCl (18 ± 3 vs 12 ± 1 ng/μg DNA/30 min). In Ca-free media KCl-induced insulin release was not observed and A-1254 evoked release was inhibited by 50%. Intracellular Ca^{++} (Indo-1 fluorescence) was increased within 40 sec of A-1254 exposure. These results indicate that PCBs and particularly non-coplanar congeners can stimulate insulin release through a process dependent, at least in part, on the entry of extracellular Ca^{++} into cells via depolarization-related channels. Further details of this process are being investigated. (NIH ES-04911).

341 EFFECT OF INSULIN-LIKE GROWTH FACTOR I (IGF-I) ON EXOCRINE PANCREATIC SECRETION

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A decrease in the pancreatic zymogen granules was observed in rats after administration of Insulin-like Growth Factor I (IGF-I). To investigate the effects of IGF-I on exocrine pancreatic secretion and compare with those of Insulin, pancreatic juice volume and outputs of protein and amylase were examined after administration of IGF-I or insulin in rats. Pancreatic juice was collected from the common bile duct every 30 min for up to 4hr under urethane anesthetization. Administration of IGF-I (15mg/kg) or insulin(10U/kg) resulted in an increase in total volumes of pancreatic juice, about 1.4 times as great as the controls. Protein output gradually decreased in the control rats, whereas it didn't change for 2.5 hr in rats administered with IGF-I or Insulin. Total protein outputs in 4 hr were 1.3 and 1.6 times as great as the controls in rats treated with IGF-I and Insulin, respectively. Changes in amylase outputs were similar to those of protein outputs. It is suggested that the decrease in the pancreatic zymogen granules after administration of IGF-I is related to the stimulation of exocrine pancreatic secretion which is observed after administration of insulin.

342 *IN VIVO* BATTERY FOR ENDOCRINE ACTIVITY

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In vivo endpoints for estrogenicity include increases in uterine fluid and vaginal cornification incidence, serum prolactin levels, uterine weight, uterine epithelial cell height, uterine stromal cell proliferation, and uterine progesterone receptor (PR) number, and decreases in uterine estrogen receptor (ER) number. Endpoints demonstrating changes in dopamine regulation include increases in prolactin and decreases in growth hormone levels. We have combined these 9 endpoints into a single 5-day *in vivo* screening procedure to identify estrogenic compounds and dopaminergic modulators, both of which play important roles in enhancing mammary tumorigenesis in rodents. The estrogen agonists estradiol (E2) and estriol (E3), the mixed estrogen agonist tamoxifen (TAM), the pure antiestrogen ICI 182,780 (ICI), and the dopamine antagonists haloperidol (HAL) and reserpine (RES) were tested using a 3 time/day (8 hour intervals) intraperitoneal dosing regimen in ovariectomized female Crl:CDBR rats. All compounds were evaluated over a range of concentrations. The estrogens E2, E3, and TAM produced dose-dependent increases in absolute uterine weight, vaginal cornification incidence, and uterine stromal cell proliferation while the dopamine antagonists RES and HAL and the antiestrogen ICI did not induce any changes in these endpoints. All compounds elicited increased serum prolactin levels and uterine epithelial cell height except the estrogen antagonist ICI. Uterine cytosolic PR levels increased with E2, E3, and TAM, but decreased in HAL-, TAM-, and RES-treated rats. Uterine cytosolic ER levels decreased with the antiestrogen ICI and the mixed agonist TAM, and were unaffected by the remaining compounds. This battery of 9 endpoints provide a "fingerprint" in which to identify compounds as being either estrogen agonists/antagonists or dopamine antagonists in a single short-term *in vivo* experiment.

343 EVALUATION OF U-96183 AS A REDUCING CO-SUBSTRATE AND MECHANISM-BASED PEROXIDASE INHIBITOR

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U-90152T is a non-nucleoside reverse transcriptase inhibitor, currently under development for the treatment of Acquired Immunodeficiency Syndrome (AIDS). In previous studies with this compound, thyroid hypertrophy/hyperplasia was observed which appeared to be secondary to decreased serum thyroxine levels. U-96183 is a major metabolite of U-90152T, which as a primary aromatic amine, could serve as a reducing co-substrate and/or mechanism-based inhibitor of thyroid peroxidase, the rate-limiting step in thyroxine biosynthesis. In these studies both lactoperoxidase, frequently used to model thyroid peroxidase, and horseradish peroxidase were used to evaluate this hypothesis. Initial spectral studies suggested that U-96183 was not a facile reducing co-substrate. Substantive metabolism of benzidine, a positive control, was observed with both enzymes. The spectral studies, however, could not distinguish between a poor co-substrate and mechanism-based inhibition. Therefore, the effects of U-96183 on the reduction of 5-phenyl-4-pentenyl hydroperoxide (PPHP) to its corresponding alcohol (PPA) were examined. Consistent with the spectral studies, only a small amount of PPHP was reduced by either enzyme in the presence of U-96183 alone. Additionally, U-96183 did not inhibit benzidine-dependent PPHP reduction, indicating that U-96183 is not a mechanism-based peroxidase inhibitor. These data are consistent with previous studies suggesting that U-90152T-dependent decreases in serum thyroxine levels are secondary to enhanced excretion rates.

344 LEVOTHYROXINE SUPPLEMENTATION BLOCKS U-90152T-DEPENDENT CHANGES IN THE RAT THYROID GLAND

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U-90152T is non-nucleoside reverse transcriptase inhibitor, currently under development for the treatment of HIV infection. In multi-dose pre-clinical

studies in rats, increased thyroid gland weights were observed, accompanied by follicular cell hypertrophy. Studies reported here examined the hypothesis that the thyroid changes were secondary to feedback stimulation of the thyroid gland by thyrotropin (TSH), secreted in response to decreased circulating levels of T_4. Rats (SD) received vehicle or U-90152T, 250 mg/kg/day, p.o., in two divided doses. Subgroups were supplemented with levothyroxine, 60 or 120 μg/day, s.c.. Administration of U-90152 for 14 days resulted a 2.2- and 2.9-fold increase in absolute thyroid gland weights in males and females, respectively. Serum T_4 concentrations were substantively reduced by U-90152, with concomitant increases in thyrotropin. Supplementation with levothyroxine returned both T_4 and TSH to control levels and blocked both the increase in thyroid gland weight and microscopic evidence of follicular cell hypertrophy. Thus, the data are consistent with the effects of U-90152T on the rat thyroid gland occurring via feedback stimulation, mediated by TSH. Hepatic microsomal UDP-glucuronosyltransferase activities v T_4 were increased 2.6-fold in males and 5.3-fold in females, suggesting that the decrease in T_4 levels are initially caused by enhanced T_4 clearance.

345 CHANGES IN THYROID HORMONE LEVELS AFTER FOURTEEN DAY ORAL-DOSING WITH AMMONIUM PERCHLORATE

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The use of ammonium perchlorate (AP), CAS registry number 7790–98–9, in the manufacture of solid rocket motors has resulted in soil and water contamination. Remediation levels proposed by the US EPA are extremely conservative because of an insufficient toxicity database. Since the thyroid is the target organ and perchlorate inhibits iodine uptake, this study evaluated the potential of AP to produce changes in levels of triiodothyronine (T3), reverse T3 (rT3), T4, and thyroid stimulating hormone (TSH), by providing specific dose-response information for use in establishing a remediation level. Groups of six male and six female Sprague-Dawley rats were dosed with AP in drinking water at concentrations of 0 (control), 1.25, 5.0, 12.5, 25, 50, 125, or 250 mg/L. Animals were sacrificed after fourteen days. T3 decreased in a dose dependent manner, and rT3 and TSH increased in a dose dependent manner for both sexes. T4 levels did not change in females but decreased in males in the two highest dose groups.

346 FORMATION OF 8-OXODEOXYGUANOSINE IN BRAIN DNA OF RATS EXPOSED TO ACRYLONITRILE

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Acrylonitrile (ACN) produces tumors in rats, but tests for genotoxicity have yielded mixed results. Gliomas were found after ACN exposure, but no ACN-DNA adducts have been identified in the brain. The 7-(2-oxoethyl)guanine adduct has been identified in rat liver *in vivo*, but liver is not a target organ for tumor production. Therefore, experiments were conducted to investigate possible epigenetic mechanisms of ACN tumorigenesis in the rat brain. Male F344 rats were exposed to 0.75 mmol ACN/kg by subcutaneous injection and were killed after 1, 3 or 6 hours. The level of 8-oxodeoxyguanosine was found to be elevated 2-fold 3 hours after exposure compared to 1 or 6 hours. In another experiment, rats were exposed to 0, 3, 30 and 300 ppm ACN in drinking water for 21 days. In the 30 and 300 ppm ACN groups, 8-oxodeoxyguanosine levels were 2-fold greater than controls. In this study, there was no change in the levels of cell proliferation of subependymal glial cells as measured by PCNA immunohistochemistry. In both experiments, measures of glutathione levels and thiobarbituric acid reactive substances were not significantly changed. These studies suggest that ACN-induced tumors are produced by a mechanism involving formation of 8-oxodeoxyguanosine, which may reflect oxidative damage.

347 FORMATION OF MALONDIALDEHYDE-MODIFIED DEOXYGUANOSINYL ADDUCT FROM METABOLISM OF CHLORAL HYDRATE BY MOUSE LIVER MICROSOMES

Y- C Ni, F F Kadlubar and P P Fu. *National Center for Toxicological Research, Jefferson, AR.* Sponsor: D W Roberts

We previously reported that metabolism of chloral hydrate (CH), a widely used sedative and hypnotic, by male $B6C3F_1$ mouse liver microsomes resulted in lipid peroxidation, producing the tumorigen malondialdehyde (MDA). We now report that metabolism of CH by mouse liver microsomes in the presence of calf thymus DNA results in the formation of an MDA-modified DNA adduct which was detected by ^{32}P-postlabeling analysis. Similar results were obtained from metabolism of trichloroacetic acid (TCA) and trichloroethanol (TCE), both metabolites of CH. Adduct formation from CH and TCA was nearly equal (about 1.1 adducts/10^6 nucleotides) while that formed from TCE was three-fold lower. This adduct formed from incubation of CH, TCA, or TCE with liver microsomes of pyrazole-treated mice was two- to three-fold higher than that from control microsomes. Our results suggest that metabolism of CH to TCA is the principal activation pathway leading to lipid peroxidation and formation of the MDA-modified DNA adduct and that CYP2E1 is a major metabolizing enzyme.

348 OXIDATIVE DNA DAMAGE LEVELS IN MAMMARY GLAND OF RATS FED VARYING AMOUNTS OF CORN OIL

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Dietary intake of corn oil has been shown in many studies to influence mammary gland tumorigenesis in rats. One mechanism behind this association is the effect dietary fat can have on endogenous levels of oxidative DNA damage. We have examined the levels of 5-hydroxymethyl-2′-deoxyuridine, an oxidized thymine residue, in the mammary gland DNA of rats fed varying levels of corn oil. After a 2 week equilibration period using a 5% corn oil diet, rats were fed 3, 5, 10, 15 or 20% corn oil for 20 weeks. The levels of 5-hydroxymethyl-2′-deoxyuridine in DNA from mammary gland were significantly lower in rats fed 3% versus 10% fat. The levels of this oxidized base were not significantly increased in rats fed higher amounts of fat. This is unlike our previous results after 2 weeks of feeding where DNA damage levels were maximal using 5% corn oil. These results indicate that there are time-dependent effects on oxidative stress by dietary fat. Although protection against DNA damage was achieved with the low-fat diets, increased fat intake above 10% oil (22% of calories from fat) did not further appreciably increase oxidative DNA damage levels. Supported by grant number NC93B61 from the American Institute for Cancer Research.

349 CARCINOGENIC AROMATIC AMINES INDUCE OXIDATIVE STRESS IN YEAST

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The carcinogenic aromatic amines aniline, o-toluidine and o-anisidine are negative or difficult to detect as mutagens in the Ames Salmonella mutagenicity assay and other short term tests. They do, however, induce intrachromosomal recombination between repeated sequences in Saccharomyces cerevisiae, resulting in deletion (DEL) of intervening sequences. We have investigated whether the generation of free radical species by these compounds or their metabolites may be responsible for their recombinagenic activity in S. cerevisiae. The free radical scavenger N-acetyl cysteine protected against the lethal and recombinagenic effects of aniline, o-toluidine and o-anisidine. Sensitivity to their lethal effects was greatly increased in strains deficient in superoxide dismutase. Intracellular oxidation of the free radical-sensitive reporter compound dichlorofluorescin to the fluorescent dichlorofluorescein in growing cultures of S. cerevisiae was induced by treatment with aniline, o-toluidine and o-anisidine. The three carcinogens were also weak inducers of the redox responsive soxRS regulon in Escherichia coli, indicating the generation of superoxide radical by these compounds. The primary products of mammalian aniline metabolism 4-aminophenol and 2-aminophenol were significantly more potent inducers of oxidative stress in yeast and E. coli, and of DEL recombination in yeast, than aniline, suggesting the importance of metabolic activation by cytochrome p450 in the xenobiotic activity of aniline. The results demonstrate a role for oxidative stress in the toxicity and recombinagenicity of these carcinogenic aromatic amines.

350 FUMONISIN B[1]-INDUCED LIPID PEROXIDATION AND DNA DAMAGE IN ISOLATED RAT LIVER NUCLEI

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Fumonisin B_1 (FB_1), a contaminant of corn, is reported to be a nongenotoxic hepatocarcinogen in rats. In an attempt to understand its mechanisms of action, a model system of isolated rat liver nuclei was used to determine what effects, if any, it might have on nuclear membrane lipids and DNA.

FB_1 induced a concentration-dependent increase of lipid peroxidation concurrent with DNA strand breaks. Iron and copper had no statistically significant stimulatory effects on these reactions. The active oxygen scavengers catalase, superoxide dismutase (SOD), mannitol and sodium azide had no significant inhibitory effects on the FB_1-induced DNA strand breaks. However, a small but significant reduction in lipid peroxidation by catalase, SOD and mannitol was observed. The results suggest that the hydroxyl radicals are initiators of nuclear membrane lipid peroxidation producing peroxyl radicals, which in turn, may be responsible for DNA strand breaks. Alternatively, the hydroxyl radicals produced near the DNA backbone may induce direct site-specific strand breaks, which are insensitive to the free radical scavengers.

351 EFFECT OF CALORIC RESTRICTION ON OXIDATIVE STRESS BIOMARKERS IN LIVER AND KIDNEY OF SPRAGUE-DAWLEY RATS

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In an ongoing two-year carcinogenicity study conducted in-house, male and female Sprague-Dawley rats are fed *ad libitum* (AL) or at moderate (70–75% of AL) or marked (50% of AL) dietary restriction (DR). In addition to toxicologic and pathologic parameters, numerous biochemical endpoints are being examined. Reported here are 4-hydroxy-2-*E*-nonenal (4-HNE), glutathione (GSH), malondialdehyde (MDA), and 8-hydroxydeoxyguanosine (8-OHdG) contents in kidney and/or liver. After 13 or 26 weeks on the feeding regimens, hepatic levels of GSH were increased up to 80% in DR animals vs AL animals. At 26 weeks, the hepatic contents of 4-HNE and MDA were reduced by as much as 70% in DR rats as compared with AL rats. However, no effect of DR on hepatic 8-OHdG content was detected. In kidney, neither 8-OHdG nor GSH levels were dramatically different between the feeding groups after 13 and 26 weeks. These early data indicate that restricting the caloric intake (DR) of rats may prevent loss of protective mechanisms and ease oxidative stress, and thus delay the onset of proliferative and degenerative lesions sufficiently to prolong the lifespan of Sprague-Dawley rats.

352 Ni(II) INHIBITS THE NUCLEOTIDE POOL SANITIZING ENZYME MutT

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MutT is an enzyme that hydrolyzes 8-oxo-dGTP, thus preventing incorporation of the promutagenic 8-oxo-dG into DNA. Since this hydrolysis is activated by Mg(II), it may be sensitive to inhibition by other divalent metals, including carcinogenic nickel. To test this hypothesis, enzyme kinetic studies of the effect of Ni(II) on mutT were conducted. Purified mutT (a gift from Dr. M. Bessman, Johns Hopkins University) was assayed by HPLC in 100 mM ammonium acetate, pH 7.4, in a model system with 4 mM dGTP, 5–34 mM Mg(II), and 0–1.5 mM Ni(II). Ni(II) inhibited the dGTP hydrolysis in a concentration-dependent manner, with 1.5 mM Ni(II) decreasing Km of mutT for Mg(II) by 68% and Vmax by 62%. The Ni(II) inhibition mechanism was determined to be uncompetitive with K_i' of 0.81 mM. This inhibition mechanism suggests that Ni(II) does not directly compete with Mg(II) for binding on mutT but that Ni(II) binds at a site distinct from that of Mg(II). Ni(II) has been shown to mediate oxidative DNA damage, the main product being 8-oxo-dG [Kasprzak, *Cancer Invest.* **13**: 411, 1995]. The present study indicates that the oxidative damage may be further potentiated by Ni(II) inhibition of mutT.

353 EFFECT OF CHLOROACETONITRILE (CAN) ON GLUTATHIONE (GSH) TURNOVER AND ITS IMPACT ON OXIDATIVE DNA DAMAGE, 8-HYDROXY-2'-DEOXYGUANOSINE (8-OHdG), IN GASTRIC MUCOSA OF RATS

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CAN, a member of haloacetonitrile group, is detected in drinking water supplies as a by-product of the chlorination processes. Gastrointestinal (GI) tract is a potential target organ of haloacetonitrile-induced acute and genetic toxicity. To examine the genotoxic mechanism of these chemicals, we studied the effect of CAN on GSH balance and its impact on oxidative DNA damage in gastric mucosal cells. Animals were treated with a single oral dose (38.3 or 76.6 mg/Kg) of CAN, sacrificed at variable times (0–24 hr) after treatment and gastric mucosal tissues were collected. CAN induced a significant, dose and time dependent, decrease in GSH levels in pyloric stomach mucosa at 2 hr after treatment (56 and 39% of a control, respectively). GSH levels rebounded to control values [3.22 ± 0.40 μmoles/g tissue] at 12 hr after treatment. The impact of the GSH depletion on oxidative damage to gastric DNA was evaluated by the electophoretic migration of genomic DNA on agarose gel and quantitated by measuring the levels of 8-OHdG in hydrolized DNA by HPLC-EC. DNA damage was observed electrophoretically at 6 and 12 hr following CAN treatment. CAN (38.3 mg/Kg) significantly elevated gastric 8-OHdG and the maximum levels of 8-hydoxylation of the guanine bases occurred at 6 hr after treatment [144.3 ± 8.9 (8-OHdG/10^5dG) 146% of control]. With high dose of CAN (76.6mg/Kg), however, peak level of 8-OHdG [174.5 ± 19.5 (8-OHdG/10^5dG) 177% of control] was observed at 2 hr following CAN treatment. These data indicate that a mechanism of chloroacetonitrile induced genotoxicity may be partially mediated by depletion of glutathione, interruption of the cellular antioxidant mechanism and induction of oxidative damage of gastric DNA. [Supported by NIH grant # ES 01871].

354 DNA DAMAGE RESULTING FROM THE OXIDATION OF CATECHOL ESTROGENS BY COPPER: COMPARISON OF 8-OXO-DEOXYGUANOSINE (8-OXO-dG) TO DNA STRAND BREAK FORMATION

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Catechol estrogens can be oxidized by Cu(II) to generate reactive O_2 species which cause single and double strand breaks in plasmid DNA. Our goal was to determine the relative sensitivity of detecting DNA strand breaks versus 8-oxo-dG adducts. In the presence of 2-hydroxyestradiol (2-OH-E_2) plus 10μM Cu(II), 8-oxo-dG and DNA strand breaks increased in a dose and time-dependent manner. DNA single strand breaks increased from 22% to 82% between 1 and 5 μM 2-OH-E_2, whereas the equivalent percentage of plasmids containing a single 8-oxo-dG increased from 10% to 45%. No increases in 8-oxo-dG or strand breaks were detectable in the presence of Cu(II) or catechol alone. Cu(I) chelation blocked both forms of DNA damage. 500U/ml catalase blocked strand breaks, but only inhibited 8-OH-dG formation by ≈20%. Hydroxyl radical scavengers, which do not inhibit strand break formation, modestly inhibited 8-oxo-dG formation. Of the singlet oxygen scavengers, TRIS and sodium azide both inhibited DNA strand break formation but enhanced 8-oxo-dG formation, whereas 2,2,6,6-tetramethly-4-piperidone inhibited 8-oxo-dG and strand break formation. Thus, DNA degradation does not directly correlate with 8-OHdG formation. (Supported by PHS grants ESO7141 and CA36701).

355 POLYAMINES AND POLYAMINE ANALOGUES PROTECT DNA FROM STRAND BREAKS INDUCED BY REACTIVE OXYGEN SPECIES

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Reactive oxygen species are known to produce single and double strand DNA breaks and are associated with mutations and programmed cell death. The natural polyamines have been suggested as being protective agents against oxidative DNA damage. Several investigators have proposed that the antitumor action of polyamine analogs is based on their interactions with DNA. We have determined the protective effects of polyamine analogs and spermine against ϕX-174 plasmid DNA strand breaks caused by reactive oxygen species generated by Cu(II) ion + H_2O_2. The polyamine analog N^1-ethyl-N^{11}-((cyclopropyl)methyl)-4,8-diazundecane at 1–6 mM and 1mM spermine reduced the appearance of strand breaks. In comparison with other antioxidants, spermine was more effective than glutathione and sodium azide, a singlet oxygen scavenger. Strand break formation was also blocked by catalase, N-acetyl-L-cysteine and bathocuproinesulfonic acid, a Cu(I) chelator. 5,5-dimethyl-1-pyrroline-N-oxide, a free hydroxyl radical scavenger, had no inhibitory effect, suggesting that the reactive oxygen species involved was not the free hydroxyl radical. Thus, polyamine analogues and spermine can protect against DNA strand breaks by reactive oxygen species. Supported by grants ES07141, CA09071, CA57545, CA63552 and CA51085.

356 DIQUAT CAUSES DNA FRAGMENTATION *IN VIVO*, BUT DOES NOT INCREASE 8-OH-dG CONTENTS

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Administration of 0.1 mmol/kg of diquat to Fischer-344 rats causes acute hepatic necrosis by mechanisms that appear to involve increased generation of reactive oxygen species (ROS). The oxidation of biological molecules should be pivotal in the expression of the injury, but the specific targets are not known. We assessed protein thiol status by derivatization of subcellular fractions with monobromobimane and separation of the fluorescent derivatives by SDS-PAGE. No differences from saline-treated controls were seen in animals 2 or 6 h after diquat, despite documentation of injury by elevated plasma transaminase activities. Hepatic DNA fragmentation (% of total) was increased in diquat-treated animals (6.7 ± 0.3 vs. 24.9 ± 5.1 at 2 h; 4.6 ± 0.3 vs. 57.2 ± 4.1 at 6 h, $P<0.001$). However, 8-OHdG contents in hepatic DNA were not increased by diquat (35.3 ± 6.2 mol 8OHdG/10^6 mol dG) over controls (28.3 ± 2.6). Plasma NH_3 concentrations increased in diquat-treated rats from 49 ± 9 in controls to 170 ± 22 μM 6 h after diquat ($P<0.002$). Hepatic glutamine synthetase activities were lower in diquat-treated rats (39.7 ± 3.0 mU/mg pro) than in controls (65.8 ± 3.4, $P<0.001$), but activities of carbamyl phosphate synthetase-I, which is a mitochondrial enzyme, were not decreased significantly. Supported by NIH grants GM44263 and GM39338.

357 FORMATION OF 8-HYDROXY-2'-DEOXYGUANOSINE IN HEART, LIVER, AND LUNG TISSUE DUE TO ENVIRONMENTAL TOBACCO SMOKE EXPOSURE

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Environmental tobacco smoke (ETS) is a hotly debated social, political, and scientific issue; pitting smoker's rights against the health and safety of non-smokers. Striking an acceptable balance between the two depends largely on the potential health hazard assessment of ETS. Studies from this laboratory have shown that exposure to ETS contributes to oxidative stress in mouse heart, liver, and lung tissues. This study measures the level of oxidative damage to mouse liver, lung, and heart DNA as a result of this oxidative stress. Adult female Balb/c mice were exposed to a regimen consisting of sequences of a 30 minute exposure followed by a 1.5 hour non-exposure, repeated up to 3 times. Tissues were excised following the exposure and non-exposure periods. DNA was then extracted from the tissues and analyzed for the presence of the oxidative product 8-hydroxy-2'-deoxyguanosine (8-OHdG). In all three tissues, the exposure increased the presence of 8-OHdG above the control levels. In some instances, the increased levels returned to normal by the end of the non-exposure period, while other tissues showed a further increase following non-exposure. These studies demonstrate that limited exposure to ETS produces measurable DNA oxidative damage. This work was supported in part by Grant #NEV 0714 from the NV. Ag. Exp. Station.

358 DETECTION OF NUCLEIC ACID-PROTEIN CROSS-LINKING AND ITS ASSOCIATION WITH 8-HYDROXYGUANINE FOLLOWING VIRAL INACTIVATION BY METHYLENE BLUE PLUS LIGHT

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Irradiation with white light (L) with Methylene blue (MB) is used as an antiviral treatment for blood products. We have shown that MB + L mediates the formation of high levels of 8-hydroxyguanine (8-OHGua) in DNA and RNA. Using the single-stranded RNA bacteriophage Qβ, MB + L mediated inactivation was associated with RNA-protein crosslinks and higher 8-OHGua levels. We present data showing that enhanced levels of 8-OHGua and other nucleoside adducts (possibly nucleoside-amino acid complexes) are formed following exposure of DNA and histone proteins to MB + L. Since nucleic acid-protein crosslink formation by MB + L has been shown to be dependent upon intercalation of MB into the nucleic acid, the observation of such crosslinks may constitute a test for the means by which MB + L may inactivate a particular virus. A lipid-containing bacteriophage, PM2, was tested for nucleic acid-protein crosslink formation mediated by exposure to inactivating levels of MB + L. Preliminary results indicate that crosslinks were formed at levels of MB + L corresponding to 90%–99% inactivation of the phage. Quantitation of crosslinking and inactivation of PM2 are in progress. This research was supported by NIH grant HL 53585.

359 MECHANISTIC DIFFERENCES IN XENOBIOTIC-MEDIATED EXPRESSION OF CYTOCHROME P450 2E1 AND 2B BETWEEN THE FGC-4 RAT HEPATOMA CELL LINE AND PRIMARY CULTURED RAT HEPATOCTYES

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CYP2E1 and 2B mRNA and protein expression were examined in FGC-4 cells and primary cultured rat hepatocytes in response to chemicals known to enhance hepatic 2E1 and 2B expression *in vivo*. Treatment (24 hr) with 100 mM ethanol (ETH) or acetone (ACE) enhanced 2E1 protein expression in hepatocytes by 4- and 3-fold, respectively, while no detectable increases in 2E1 protein were observed in FGC-4 cells, compared to untreated cells. Corresponding 2E1 mRNA levels were decreased in hepatocytes to 54% (ETH) and 75% (ACE) of the levels in untreated cells. In FGC-4 cells, however, ETH decreased 2E1 mRNA levels by 25%, while ACE produced a 2-fold increase. Pyridine (PYR) treatment (10 mM) resulted in a 2- to 3-fold increase in 2E1 protein levels in both hepatocytes and FGC-4 cells. While this treatment did not alter 2E1 mRNA levels in hepatocytes, a 3-fold increase in 2E1 mRNA was detected in FGC-4 cells compared to untreated cells. CYP2B expression was also affected differentially by xenobiotics in hepatocytes versus FGC-4 cells. CYP2B mRNA and protein expression in FGC-4 cells were either unchanged or increased only slightly (1.5- to 2.5-fold) by treatment with ACE, PYR, or 0.1 mM phenobarbital (PB). In contrast, 2B mRNA levels were increased approximately 36-, 27-, and 30-fold by ACE, PYR, and PB, respectively, in primary cultured hepatocytes, with corresponding increases in 2B protein levels of approximately 55-, 18-, and 17-fold (ACE, PYR, and PB, respectively). These data show that the expression of 2E1 and 2B in response to xenobiotics in FGC-4 cells differs mechanistically from that observed in primary cultured rat hepatocytes, the latter paralleling that reported *in vivo*. Supported by NIEHS grants ESO3656 and P30 ESO6639.

360 IN VITRO METABOLIC ENZYME INDUCTION: MEASUREMENT OF mRNA IN H4Ile RAT HEPATOMA CELLS AFTER XENOBIOTIC TREATMENT

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Hepatic enzyme induction is often evoked as the cause for a variety of changes at the whole animal and tissue level, including hepatic hypertrophy, increased drug clearance, or increased toxicity, after a repeated treatment regimen. Enzyme induction has as its basis a molecular response: a compound interacts with a receptor protein which then directly or indirectly increases the synthesis, stability or translation into protein of the messenger RNA coding for a particular metabolic enzyme. In some cases the stability of the enzyme protein itself is increased. Being a molecular event, metabolic enzyme induction should lend itself well to modeling and study in vitro. We describe here experiments using the H4Ile rat hepatoma cell line thats suggest that this cell line is capable of accurately modeling metabolic enzyme induction. Cells cultured on Primaria® treated dishes respond to treatment with 25 nM dexamethasone or 0.1 mM phenobarbital (PB) by an increase in mRNA for Cyp2B1 as measured by a semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assay. They similarly respond to aminoglutethimide, an aromatase inhibitor and known inducer of P450 2B1 *in vivo*. As has been shown *in vivo*, Cyp2B2 mRNA does not appear to be substantially increased. The semi-quantiative RT-PCR assay readily detects mRNA for family 2 UDP-glucuronyl transferases. With the addition of a quantitative RT-PCR assay, and assays for enzyme activity, the system would offer the promise of a convenient in vitro metabolic induction assay.

361 DETECTION OF CYTOCHROME P450 (CYP) mRNAs IN TISSUE AND CELL LINES USING ENZYME-LABELED FLUORESCENCE *IN SITU* HYBRIDIZATION

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CYPs constitute a multigene superfamily of enzymes that metabolize an array of xenobiotics. Measuring basal and potential induction of P450 mRNA levels in specific cells and tissues upon exposure to drugs and other xenobiot-

ics should provide valuable insight regarding the functional role of these enzymes in chemically-related diseases. Prior methodologies for detection of cell-specific transcriptional activity patterns have utilized radiolabeled probes, often coupled with long exposure times. These studies were conducted to evaluate an Enzyme-Labeled Fluorescence (ELF) *in situ* hybridization technique to detect specific CYP mRNAs. Deparaffinized, formalin-fixed tissue sections or cells from culture were incubated with 5'-biotinylated 20 base pair oligomer probes for 12 hours. Specific hybridization was detected using a streptavidin alkaline-phosphatase conjugate followed by incubation with the ELF substrate, yielding a bright, yellow-green fluorescent signal. This technique eliminates the use of radioactivity and enables detection of mRNAs with relative ease, efficiency, specificity, and high levels of sensitivity. Our results clearly demonstrated that ELF *in situ* hybridization can be employed to discriminately amplify and detect cell-specific P450 mRNA expression levels in both tissue sections and cultured cells. The results obtained using the ELF technique correlated well with other mRNA detection methods such as Northern blotting and reverse transcriptase coupled polymerase chain reaction analyses. Supported by NIH Grant ES-04696.

362 DETERMINATION OF CYTOCHROME P450 ISOFORMS BY RT-PCR AND WESTERN BLOTS IN ADULT RAT HEPATOCYTES CULTURED ON MATRIGEL

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Reverse transcriptase-polymerase chain reaction (RT-PCR) and immunochemical techniques were used to detect and quantitate the expression of cytochrome P450 (CYP450) isoenzymes in primary cell cultures. Adult rat hepatocytes were cultured on Matrigel in a serum-free medium supplemented with insulin for three days. CYP450 inducers such as phenobarbital (PB, 2×10^{-3} M), hydrocortisone hemisuccinate (HC, 1×10^{-4} M), clofibrate (CLO, 2×10^{-3} M), and 3-methylcholanthrene (3MC, 2×10^{-6} M), were added to the cell cultures on days two and three as concentrated stock solutions. Several isoforms of CYP450 (2B1, 3A1, 4A1, and 1A1) were assessed. Specific PCR primers were designed and used to detect the mRNA expression of each CYP450 isoenzyme under optimized linear amplification conditions. For immunoblotting, mono-and polyclonal antibodies selective for the CYP450 isoenzymes were obtained from commercial sources and used for detection of protein levels in cell lysates. Analysis of CYP450 expression in cultured hepatocytes revealed that the major isoforms induced by PB, HC, CLO, and 3MC were 2B1/2, 3A1, 4A1 and 1A1, respectively, and were in agreement by the two detection techniques. The results from this study have demonstrated the utility of RT-PCR for the analysis of CYP450 isoenzyme expression in rat hepatocytes following exposure to xenobiotics. In addition, RT-PCR is proven to be a sensitive, fast, and reliable method with applications in all areas of toxicology.

363 PRECISION-CUT RAT LIVER SLICES AS A MODEL TO INVESTIGATE INDUCTION OF CYTOCHROME P450-DEPENDENT ENZYMES

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The effects of reference inducers, 3-methylcholantrene (3-MC), dexamethasone (DEX) and phenobarbital (PB) on cytochrome P450-dependent enzymes were studied in precision-cut rat liver slices prepared from male Sprague Dawley rats. The slices (250-280μm) were obtained using the Krumdieck tissue slicer and incubated for 72 hours in Williams' medium E in the absence or presence of an inducer in a dynamic rotating culture system in an atmosphere of 95:5% (air: CO_2). 7-ethoxyresorufin-*O*-deethylase (EROD-CYP1A1), 7-pentoxyresorufin-*O*-depenthylase (PROD-CYP2B1/2), ethylmorphine *N*-demethylase (EMND-CYP3A) were measured fluorometrically and hydroxylation of testosterone was quantified by HPLC method in microsomal fractions obtained from slices. 3-MC (5gmM) increased EROD activity (1.5 fold at 48 hr and 72 hr) but not that of PROD and EMND. DEX (1gmM) only enhanced EMND activity (9 and 6-fold at 48 hr and 72 hr, respectively). However, PB (2mM) did not modify these three enzyme activities. The hydroxylation of testosterone related to CYP3A (2gb, 15gb, 6gb hydroxytestosterone) was enhanced by DEX while no effects were detected with 3MC. The level of cytochrome P4501A1 protein assessed by Western blot analysis was increased by 3MC suggesting that the rise in EROD activity is related to an increase in CYP1A1 synthesis. In conclusion, in our experimental conditions, liver slices appear to be a suitable in vitro model for studying induction properties of compounds on cytochrome P450-dependent enzyme activities.

364 COMPARISON OF BASAL EXPRESSION AND INDUCTION PROFILES OF BIOTRANSFORMATION ENZYME SYSTEMS IN PRIMARY AND TRANSFORMED RAT HEPATOCYTE CULTURES

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In this study we characterized four transformed rat hepatoma cell lines, C2Rev7, H4IIE, Fao and FGC-4 relative to primary rat hepatocyte cultures, with respect to various markers of liver-selective and biotransformation enzyme expression. Xenobiotic induction responses also were evaluated using dexamethasone, β-naphthoflavone, and phenobarbital (PB). With standard mRNA hybridization, protein immunochemical and enzymatic assays, inducible and basal levels of expression for most genes in biotransformation enzyme battery were readily detected in primary hepatocyte cultures. However, more sensitive techniques, such as reverse transcriptase-coupled PCR (RT/PCR) and *in situ* hybridization analyses using enzyme-linked fluorescence (ELF) often were required for detection of these profiles in the cell lines examined. Comparitively, the FGC-4 and Fao lines exhibited the highest levels of gene expression for CYP2B1, 2B2, 2E1, and 3A1. All lines displayed high levels of uninducible microsomal epoxide hydrolase and glutathione-*S*-transferase *Pi*. However, the expression of the liver-selective albumin gene was poorly correlated with the capacity of the cell lines to respond to xenobiotic inducers. Supported by NIH grants GM32281 and ES04696.

365 INDUCTION OF CYP GENE EXPRESSION BY P-HYDROXYPHENOBARBITAL AND P-HYDROXYPHENYTOIN IN PRIMARY RAT HEPATOCYTES

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In vivo studies have suggested that the induction of CYP2B gene expression in rat liver by phenobarbital (PB) is associated with the parent molecule and not with its major hepatic metabolite, *p*-hydroxy PB (PB-OH). Since this may be due to the poor bioavailability and rapid dearance of the metabolite *in vivo*, we compared the induction of CYP gene expression by PB and PB-OH in a primary rat hepatocyte culture system, involving an ECM overlay, that dosely mimics the PB-mediated *in vivo* CYP induction. Examination of total RNA and protein for induction of CYP gene expression using slot-, northern-, and western-blotting analyses showed that 10-20-fold greater concentrations of PB-OH were required, relative to PB, for similar induction of CYP2B1, 2B2 or 3A1. While CYP2B1 induction was saturated at 0.5 mM, 2 mM PB-OH was required for significant CYP3A1 induction. PB-OH, but not PB, induced CYP1A1 and 1A2 mRNA and protein at concentrations >0.5 mM. Similar results were obtained with the p-hydroxy metabolite of phenytoin. These results indicate that there are potent concentration-dependent induction effects associated with the p-hydroxy metabolites of PB and phenytoin that have previously not been detected in *in vivo* studies. Supported by NIH grant GM32281 (CJO,JSS).

366 MECHANISTIC STUDIES ON THE INDUCTION OF RAT LIVER AROMATASE ACTIVITY BY THE PEROXISOME PROLIFERATOR AMMONIUM PERFLUOROOCTANOATE (C8)

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The incidence of Leydig cell adenomas increases in CD rats fed for 2 years with the hepatic peroxisome proliferator, ammonium perfluorooctanoate (C8). Treatment with C8 increased the serum concentration of estradiol in 2 week gavage studies, and feeding studies at time points of up to 2 years. This increase in serum estradiol was significantly correlated with the increase in total hepatic aromatase activity (cytochrome P450–19), the cytochrome P450 monooxygenase which converts androgens to estrogens. The present study investigated the mechanism by which C8 increases hepatic aromatase activity. Aromatase activity was measured in rat liver microsomes by the release of tritiated-water from [1β-3H]androstenedione. Co-incubation of liver microsomes with different concentrations of C8 during the assay significantly reduced the apparent aromatase activity. This decrease in aromatase *in vitro* despite the observed increases *in vivo* was further investigated using cultured

rat hepatocytes. C8 treatment produced a biphasic response, dose-dependently reducing hepatocyte aromatase activity after 18 and 42 hours, and increasing the enzyme activity by almost 2-fold after 66 hours of treatment, a phenomenon characteristic of enzyme induction. Lineweaver-Burk analysis of aromatase enzyme kinetics ± 20 μM C8, at different androstenedione concentrations, showed that C8 reduced Vmax without affecting Km, indicating that the initial inhibition of aromatase was non-competitive in nature. Spectral binding studies showed that C8 interacted with microsomal cytochrome P450 in a manner consistent with Type I and not Type II binding characteristics. The results of these studies suggest that the increased hepatic aromatase activity produced by C8 in rats is due to a direct effect on the liver to induce aromatase cytochrome P450 in the endoplasmic reticulum.

367 3-AMINOBENZAMIDE, A MODULATOR OF APOPTOSIS AND NECROSIS INDUCED BY ACETAMINOPHEN DOES NOT INTERFERE WITH P450IIE1 ACTIVITY *IN VIVO* OR *IN VITRO*

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Our previous studies indicate that 3-aminobenzamide (3-AB), a potent modulator of DNA repair, exerts a remarkable cytoprotection against acetaminophen hepatotoxicity including blocking both the cell death processes- apoptosis and necrosis (Yahya, et al., 1995). As the majority of investigators consider DNA repair enzymes to be the primary target of 3-AB, its effects on drug metabolizing enzymes are not known. Objectives of the present study were two fold: (i) to determine the effects of 3-AB, and other cytoprotective agents, e.g. cholesteryl hemisuccinate (CS) and diltiazem on *in vitro* aniline hydroxylation; and (ii) to determine whether 3-AB alters catalytic activity of P450IIE1 *in vivo*. In order to assess these effects, induced (1% acetone + 12h starvation) and uninduced mouse and rat liver microsomes were prepared and assayed for aniline hydroxylase activity. To determine *in vivo* effects, fed male ICR mice were treated with a cytoprotective dose of 3-AB (400 mg/kg,ip) and the hepatic microsomes used. *In vitro* aniline hydroxylation measurements indicated that 3-AB (0.5 /5 mM), CS (0.2 / 2 mM) or diltiazem (0.2 /2 mM) had no effect on the P450IIE1 activity. Minimal inhibitory effect (~10%) was observed only at very high concentration (non-physiologic and non-cytoprotective) range, i.e. 5–10 mM. Hepatic microsomes isolated from the *in vivo* 3-AB treated mice demonstrated no alteration in the aniline hydroxylase activity (Con: 0.65 Vs. 3-AB: 0.61 nmol/min/mg protein). These results support our earlier findings that the primary modulatory sites of 3-AB remain DNA-repair enzymes rather than P450, indicating thereby a powerful protective effect on both necrosis and apoptosis.

368 EFFECT OF P-CHLOROTOLUENE AND ITS METABOLITES ON RAT HEPATIC AND PULMONARY CYTOCHROME P4502B1 ACTIVITY

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p-Chlorotoluene (PCT) is a volatile organic solvent. Discrepancies between observed effects on cytochrome P450 activity/content have been observed depending on whether rat liver and lung microsomes are exposed to PCT *in vitro* or *in vivo*. We investigated the possibility that the metabolites of PCT may be responsible for these differences as has been suggested for p-xylene. In the preliminary experiments, concentrations of PCT and its Phase I metabolites which caused 50% inhibition in CYP2B activity (IC_{50}) were studied. Hepatic and pulmonary CYP2B activity was measured through the O-dealkylation of benzyloxyresorufin (BROD) following 15-minute *in vitro* incubations with PCT or its metabolites, p-chlorobenzylalcohol, p-chlorobenzylaldehyde, and p-chlorobenzoic acid. p-Chlorobenzoic acid did not inhibit BROD activity, therefore, was not further investigated. p-Chlorobenzylalcohol only produced linear inhibition in the lung, but not in the liver. In subsequent experiments, hepatic and pulmonary IC_{50}s were used for PCT and p-chlorobenzylaldehyde. Enzyme kinetic analyses (i.e., Eadie-Hofstee or Lineweaver-Burke plots) were used to characterize the type of inhibition observed. In the liver, uncompetitive inhibition was observed with PCT and p-chlorobenzylaldehyde. In the lung, noncompetitive inhibition was observed with PCT. p-Chlorobenzylalcohol produced uncompetitive inhibition in the lung while p-chlorobenzylaldehyde behaved primarily as a noncompetitive inhibitor. The kinetic analyses therefore suggest that PCT and its two Phase I metabolites have potent inhibitory effects on BROD activity in the lung and to a lesser extent in the liver. This enzymatic inhibition may in part be the result of microsomal metabolism of PCT.

369 REGULATION OF CYP1B1 IN MOUSE LIVER BY ACENAPHTHYLENE AND PIPERONYL BUTOXIDE

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CYP1B1, a newly found isozyme of cytochrome P450 in rodents, is constitutively expressed in some endocrine organs and induced in liver and kidney by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) which has a high affinity for the aromatic hydrocarbon receptor (AhR). Acenaphthylene and piperonyl butoxide are known to induce CYP1A2 by AhR independent mechanism(s). In order to study regulational mechanisms of this isozyme in mouse liver, both chemicals dissolved in corn oil were injected intraperitoneally into C57BL(Ah^b) and DBA(Ah^d) male mice. The northern blots show a low level of constitutive expression of CYP1B1 in liver. The CYP1B1 mRNA of treated animals' livers is induced to a higher level in DBA compared to C57BL. In contrast, 3-methylcholanthrene is a more potent inducer in C57BL. These results suggest that there exists a variety of regulatory mechanisms involved in the induction of this isozyme, a possibility currently under investigation.

370 INDUCTION OF ALTERNATIVE SPLICING OF CYTOCHROME P4502B2 IN RAT LIVER BY *IN VIVO* EXPOSURE TO ALKYL-METHYLENEDIOXY-BENZENES

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We have previously demonstrated n-alkyl-methylenedioxybenzenes (MDBs) to be effective inducers of CYP1A, 2B and 3A in rodent livers, *in vivo* or *in vitro* with cultured hepatocytes. Anti-CYP2B1/2 western blots of Sprague Dawley (SD) rat liver microsomes from animals treated *in vivo* with MDBs of varying side chain length revealed a distinct structure-activity relationship for induction of CYP2B1 and CYP2B2. A third anti-CYP2B immunoreactive band was also observed in western blots of animals treated with specific MDBs. This third polypeptide migrated slightly slower than CYP2B2, and the intensity of immunostaining was correlated with that of CYP2B2 but not CYP2B1. This novel 2B-like polypeptide was found to be an alternatively spliced variant (2B2v) of CYP2B2 containing an additional 8 amino acids (translated from 24 bp of CYP2B2 intronic sequence). This band co-migrates with authentic CYP2B2v and is not detected in anti-CYP2B immunoblots of Marshall or Wistar Munich rat liver microsomes prepared from MDB-treated animals. These rat strains are reported to have a deletion of the CYP2B2 gene, and would therefore be incapable of forming the alternatively spliced CYP2B2v polypeptide. A polyclonal antibody against a peptide corresponding to the 8 amino acid intronic insert plus several exonic residues recognizes only the CYP2B2v polypeptide on immunoblots of MDB-treated SD rat liver microsomes.

371 EXPRESSION OF CYTOCHROME P450 3A7 IN *Escherichia coli*, INFLUENCE OF MINOR N-TERMINAL CHANGES ON EXPRESSION OF RECOMBINANT HUMAN P450 3A ENZYMES

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Human cytochrome P450 3A7 was expressed using the pCW vector in *E.coli* strain DH5α using techniques which we have used previously for the related human forms, P450 3A4 and 3A5 (Gillam *et al.* 1993, 1995). Four alternative constructs (#1–4) were prepared with the following N-terminal amino acid sequences introduced by PCR-based mutagenesis:

#1: MALLLAVFL I L L Y L Y G T R T H G L F K K L -
#2: MALLLAVFL V L L Y L Y G T H S H G L F K K L -
#3: MALLLAVFL V L L Y L Y G T R T H G L F K R L -
#4: MALLLAVFL V L L Y L Y G T R T H G L F K K L -

P450 3A7 holoenzyme was expressed at low levels in all four variants, relative to expression of recombinant P450s 3A4 and 3A5, however yields of up to 50nmol/l culture were obtained in membranes from cells expressing 3A7#3. No significant holoenzyme expression could be detected without the addition of δ-aminolevulinic acid to the culture media. Recombinant P450 3A7#3 was partially purified by ion-exchange chromatography. Since 3A4,

3A5 and 3A7 differ at 8 nucleotide positions (4 amino acid changes) over the first 26 codons of the recombinant proteins, we mutated the N-termini of 3A4 and 3A5 to determine whether specific residues found in the 3A7 terminus affected expression. The N-terminus corresponding to variant #3 (above) produced high levels of hemoprotein for each recombinant form relative to other variants of the same form, while the N-terminus of variant #1 above appeared consistently to suppress expression.
Gillam *et al.*, Arch. Biochem. Biophys., 305, 123-131, 1993 Gillam *et al.*, Arch. Biochem. Biophys., 317, 374-384, 1995.

372 EFFECTS OF TUMOR-PROMOTING DOSES OF DIAZEPAM AND OXAZEPAM ON LIVER WEIGHT AND CYTOCHROME P450 IN MICE AND RATS

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Diazepam and oxazepam are benzodiazepines widely used as tranquilizers and anxiolytic drugs. Because they have fewer side effects and reduced potential for physical dependency, benzodiazepines have largely replaced barbiturates as sedativehypnotic drugs. At high doses (*e.g.*, 2500 ppm in the diet), diazepam and oxazepam have been implicated as liver tumor promoters in rats and mice, a property shared by phenobarbital. The purpose of this study was to ascertain whether diazepam and oxazepam, when fed to rats and mice at doses known to promote liver tumor formation, cause an increase in liver weight and an induction of cytochrome P450 (particularly the CYP2B enzymes). B6C3F1 mice and SD rats were fed diets containing oxazepam or diazepam at 0, 25, 125, 2500 and 10,000 ppm for ten days. Animals treated with 100, 300, 500, 1000 and 1500 ppm phenobarbital served as positive controls. Diazepam and oxazepam both caused a dose-dependent increase in liver weight (up to 77%), which was comparable to the maximal effect of phenobarbital (80% increase). The higher doses of diazepam (5000 and 10,000 ppm) and the higher doses of oxazepam (2500 ppm and above) were toxic to rats and mice, which lost ~20% or more of their body weight by the end of the 10-day treatment. Treatment of rats and mice with 125 ppm diazepam and oxazepam (which achieve blood levels comparable to therapeutic blood levels in humans) caused no changes in liver or body weight. Livers frozen in liquid nitrogen at the end of the 10-day treatment period are currently be used to prepare microsomes to evaluate the effects of diazepam and oxazepam on P450 enzyme levels. Supported by NIH grant GM37044.

373 INACTIVATION OF MOUSE CYTOCHROME P-450 2B ENZYMES (Cyp2b) BY AMINE METABOLITES OF MUSK XYLOL

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Musk xylol (MX; 1,3,5-trinitro-2-*t*-butylxylene) is a non-genotoxic mouse liver carcinogen. MX also increases Cyp2b protein levels, without increasing enzyme activity. To study the mechanism of inhibition, mice were dosed orally with [14C]-MX (200 mg/kg), and covalent binding to microsomal proteins was assessed. In naive mice, about 3% of [14C]-MX equivalents in the microsomal fraction bound covalently to protein. With phenobarbital (PB) induction, covalent binding increased 7-fold, and MX decreased PB-induced enzyme activity by 90%. However, when MX was dosed to mice receiving antibiotics to eliminate intestinal flora, no covalent binding was detected in microsomes from naive or PB-induced mice, suggesting that amine metabolites of MX produced the covalent binding. Two amine metabolites of MX, p-NH_2-MX and o-NH_2-MX (denoting the amine position relative to the t-butyl group), were synthesized to study enzyme inhibition kinetics. MX and the amines were mixed inhibitors of Cyp2b activity, with k_i values of 10^{-7}, and 10^{-8} for parent MX and metabolites, respectively. MX and o-NH_2-MX did not inactivate the enzyme, but p-NH_2-MX produced a time- and NADPH-dependent loss of Cyp2b enzyme activity. At 0.1 μM, k_{inact} for p-NH_2-MX was about 2.5 min^{-1}. Thus, MX induces Cyp2b, but p-NH_2-MX selectively inactivates the enzyme. Induction and inactivation occur at dosages of MX that far exceed human exposure. However, induction of Cyp2b may be involved in the hepatocarcinogenicity of MX.

374 COMPARATIVE PHARMACODYNAMICS OF RAT HEPATIC CYP2B INDUCTION BY DDT AND ITS METABOLITES, DDE AND DDD

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In this study we characterized the pharmacodynamics of rat hepatic CYP2B induction by the pesticide DDT [1,1,1-tri-chloro-2,2-*bis*(*p*-chlorophenyl)ethane] and its metabolites DDE[1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethylene], which is bioretained, and DDD [1,1-dichloro-2,2-*bis*(*p*-chloro-phenyl)ethane], which is metabolized further and therefore less prone to bioaccumulate. DDT, DDE and DDD were each pure phenobarbital-type P450 inducers, causing induction of CYP2B and CYP3A, but not CYP1A. The ED_{50} values for CYP2B induction (benzyloxyresorufin *O*-dealkylation) by DDT, DDE and DDD were, respectively, 103, 88 and ≥620 ppm in diet (14 days exposure). The E_{max} values for CYP2B induction by DDT, DDE and DDD were 24-, 22- and ≥21-fold, respectively, compared to control values. The potencies of the three congeners for CYP2B induction were similar, with EC_{50} values (based on total serum DDT equivalents) of 1.5, 1.8 and ≥0.51 μM, respectively. These results indicate that the intrinsic CYP2B-inducing abilities of DDT, DDE and DDD are similar, despite marked differences in bioretention.

375 INDUCTION OF LIVER MICROSOMAL CYTOCHROME P450 BY β-IONONE IN SPRAGUE DAWLEY RATS

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The inductive effects of β-ionone (BI) on liver microsomal cytochrome P450 (P450) has been previously demonstrated in mice. BI has been used as a model inducer of P450 in studying the role of metabolic activation in cocaine-induced hepatotoxicity and immunosuppression. In the present study, the effects of BI on P450 induction were investigated in Sprague Dawley rats, which are one of the most useful animal models in studying drug metabolism and chemical-induced toxicity. Administration of BI at 300, 600 and 1200 mg/kg subcutaneously 72 and 48 hr before sacrificing the animals not only significanly induced the liver microsomal activities of ethoxyresorufin O-deethylase, pentoxyresorufin O-dealkylase and aminopyrine N-demethylase, but also clearly induced the levels of P450 1A1/2, P450 2B1/2 and P450 2C proteins in liver microsomes. Meanwhile, the activities of p-nitrophenol hydroxylase and erythromycin N-demethylase were not induced in liver microsomes by BI administration. The induction of P450 enzymes by BI was much greater in male rats than was in female rats. The slot and Northern blot analyses showed that the mRNA levels of P450 1A1 and P450 2B1 were increased by BI administration, and that the level of P450 2B1 mRNA was increased from 6 hr after treatment with BI in male rats and from 12 hr after treatment in female rats. Taken together, the present results indicated that BI may also induce P450 enzymes in Sprague Dawley rats as observed in mice, and that the induction of P450 by BI may be regulated by the accumulation of mRNAs. (Supported by grants from KRICT and the Ministry of Science and Technology, Korea).

376 SUICIDE INHIBITION OF CYP2F2, THE ENZYME RESPONSIBLE FOR NAPHTHALENE (NA) METABOLISM TO A CLARA CELL TOXICIANT

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Naphthalene (NA) is metabolized by CYP2F2 in mouse lung to derivatives that are cytotoxic to Clara cells. Although the role of *in situ* metabolism has been established, considerable evidence suggests that the liver may be important in the generation of metabolites which exacerbate toxicity. The current studies were done to explore several possible suicide inhibitors of 2F2 which, by virtue of their selectivity for pulmonary P450, could be utilized as tools for discriminating pulmonary and extrapulmonary metabolism in reactive metabolite binding and toxicity. Accordingly the inhibition of NA metabolism by 1- and 2-ethynylnaphthalene (EN), 9-ethynyl-phenanthrene (EP) and 5-phenyl-1-pentyne (PP) have been investigated in lung and liver microsomes, airway explants and *in vivo*. In airway explants, PP is the most potent inhibitor followed by EP, and 1- and 2-EN. Inhibition by PP is dependent upon NADPH, the preincubation time and concentration of inhibi-

tor. Formation of 1R,2S-epoxide is more strongly inhibited than the 1S,2R isomer. Concentrations of 5 μM PP result in 80% inhibition of 1R,2S-epoxide formation in lung microsomes but only 25% inhibition in hepatic microsomes. Administration of 50 μmoles/kg PP ip resulted in decreased NA metabolism in airway (70% inhibition) but not in trachea or parenchyma. Liver microsomal metabolism was inhibited by less than 30%. These studies suggest that PP may be a useful tool for selectively altering pulmonary metabolism of naphthalene and for examining the active site of CYP2F2 Supported by NIEHS 04311, ES 06700 and 05707 and NIH-CA 38192.

377 BENZENE METABOLISM AND TOXICITY IN TRANSGENIC CYP2E1 KNOCKOUT MICE

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It is widely accepted that *in vivo* bioactivation of benzene is responsible for the myelotoxicity and leukemogenicity associated with benzene exposure. Moreover, cytochrome P450 monooxygenases, principally the P450 2E1 isoform, are implicated in *in vitro* and *in vivo* benzene metabolism. The present pilot study further investigated the involvement of CYP2E1 in the *in vivo* metabolism of benzene and in the pathogenesis of benzene-induced cytotoxicity. For the metabolism studies, male transgenic CYP2E1 knockout (KO) mice and C57BL6/N (control mouse strain from which CYP2E1 KO mice were derived) were exposed to a toxic dose of benzene (200 ppm) by inhalation, along with a radiolabeled tracer dose of ^{14}C-benzene (1 Ci/mol) for 6 hr. Urine was collected for 48 hrs post-exposure. Total urinary radioactivity (as a measure of benzene metabolism) was quantified by liquid scintillation spectrometry. For the toxicity studies, CYP2E1 KO and C57BL6/N mice were exposed by inhalation to 0 ppm (air-exposed) or 200 ppm cold benzene for 6 hr/day for 5 days. On day 5, bone marrow, thymus and spleen were removed for evaluation of cytotoxicity (cell counts) by Coulter Counter. Total urinary radioactivity was reduced from 124 nmol-eq to 20 nmol-eq (i.e., 16%) in CYP2E1 KO mice compared to C57BL6/N mice. After benzene exposure, there were no differences in cytotoxicity in any tissues in CYP2E1 KO mice compared to their air-exposed controls. However, in benzene-exposed C57BL6/N mice, the number of cells in bone marrow, thymus and spleen decreased significantly to 34%, 16% and 42%, respectively, compared to air-exposed C57BL6/N controls. Thus, in mice which lack the expression of CYP2E1, benzene metabolism and toxicity is greatly reduced. These studies conclusively demonstrate that CYP2E1 is a major enzyme involved in *in vivo* benzene metabolism and that metabolism by this P450 isoform is important for the development of benzene-induced toxicity.

378 DICHLORODIPHENYLTRICHLOROETHANE (DDT) PREFERENTIALLYINDUCES 7-BENZYLOXYRESORUFIN O-DEALKYLASE (BROD) AND 7-PENTOXYRESORUFIN O-DEALKYLASE (PROD) IN RAT LIVER

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The purpose of this study was to assess the effect of treatment with technical grade DDT on the activities of rat liver enzymes responsible for alkoxyresorufin O-dealkilation and hydroxylation of p-nitrophenol. Activities were studied in microsomes obtained from male Wistar rats (200–250 g). Single doses (0, 0.1, 1.0, 5.0 10.0 or 100.0 mgDDT/kgbw) were dissolved in corn oil and administered by gavage. Animals were sacrificed 24 h after treatment, their livers extracted and processed to obtain microsomes. Dose-dependent increases in total citochrome P-450 (CYP) levels and the activities of BROD, PROD, ethoxyresorufin O-deethylase (EROD) and p-nitrophenol hydroxilase (PNFH) were observed. Total CYP levels reached twice the control value at the highest dose. The more pronounced effects were on PROD and BROD activities, which were significantly increased at doses of 1 mgDDT/kgbw and reached their maximum response after 10 mgDDT/kg (38- and 19-fold, respectively). EROD and PNFH activities also showed significant but less pronounced increases (2.4- and 2-fold, respectively). No apparent effects on 7-methoxy O-resorufin demethylase (MROD) activity were detected. The dose of 0.1 mgDDT/kg produced no apparent effects on the activities of all enzymes studied. These results suggest that single low doses of DDT preferentially induces CYP2B1 (PROD) and CYP2B2 (BROD), indicating a phenobarbital-type induction. Supported by The John D. and Catherine T. MacArthur Foundation.

379 2,4,5,2',4',5'-HEXACHLOROBIPHENYL (2HCB) INDUCES RAINBOW TROUT HEPATIC ARYL HYDROCARBON HYDROXYLASE (AHH) AND INCREASES EXPRESSION OF ONE OF TWO CYTOCHROME P450 (CYP) 1A mRNAs

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Negligible aryl hydrocarbon receptor (AhR)-mediated responses occur after exposures of rodents to di-ortho polychlorinated biphenyls (PCBs) such as 2HCB. Induction of CYP1A1 is perhaps the most extensively characterized AhR-mediated response. We previously reported induction of hepatic ethoxyresorufin-O-deethylase (EROD) in rainbow trout 4 weeks after ip injection with 50 and 250 mg 2HCB/kg (Toxicologist 15:1052). Mass spectrometry revealed 0.4% 2, 3, 3', 4,4'- and 0.5% 2,3,4,4', 5-pentachlorobiphenyls (mono-ortho PCBs) as the only contaminants in this 2HCB stock. We now report that 2HCB increases expression of CYP1A2 but not CYP1A1 in rainbow trout liver (nomenclature from Arch. Biochem. Biophys. 310:187). Diet containing 0, 5, or 20 ppm 2HCB from the above stock was fed to 10–20 g rainbow trout for 4, 8 or 12 weeks. There was a positive correlation between hepatic 2HCB, but not mono-ortho PCB, concentration and microsomal AHH. We obtained a second stock of 2HCB which negligibly altered AhR-mediated responses in mice (Toxicol. Appl. Pharmacol. 91:56) and repeated time/concentration combinations which induced EROD with the original 2HCB stock. 2HCB increased rainbow trout hepatic CYP1A2 (up to 3.5-fold) but not 1A1 mRNA. Regulation of CYP1A2 mRNA expression was more sensitive to 2HCB than that of CYP1A1, or CYP1A2 responded to 2HCB independently of CYP1A1. Either explanation was consistent with the hypothesis that trout CYP1A2 was an ortholog of mammalian CYP1A2. (Supported by USPHS grant ES-05543).

380 EFFECTS OF DIETARY FAT AND CHRONIC ETHANOL TREATMENT ON EXPRESSION OF CYP 3A IN DIFFERENT SEGMENTS OF SMALL AND LARGE INTESTINE OF MALE RATS

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Cytochromes P450 belonging to the CYP 3A family account for up to 25% of the total cytochrome P450 present in adult human liver and also account for a majority of P450 present in human small intestine. They catalyze 6β-hydroxylation of steroids, including cortisol, testosterone and estradiol. In addition they are responsible for metabolism of drugs such as cyclosporine A. Diet plays a major role in regulating expression of P450 isozymes in small intestine. In the current study we examined the effects of dietary fat and ethanol known to affect expression of cytochromes P450 in the liver, on expression of CYP 3A in small and large intestine of male rats. Groups of 300 g male Sprague-Dawley rats (N=4–6) were fed either standard rat chow ad libitum or infused with defined diets via Total Enteral Nutrition (TEN) using corn oil (5% and 42%) for 35 days. In some TEN diets, 32% of carbohydrate calories were replaced by ethanol. Following sacrifice, duodenum, jejunum, ileum and colon were dissected from intestine and microsomes were prepared by differential ultracentrifugation. Western blot analysis using a polyclonal rabbit anti-rat CYP 3A1 antibody indicates presence of two CYP 3A apoproteins in the duodenum and jejunum, the upper band of which co-migrates with CYP 3A1 in DEX-induced liver microsomes. In ileum and colon only one CYP 3A form was detected corresponding to the upper band in duodenum. Altering levels of dietary fat (5% and 40%) did not have any significant effects on expression of CYP 3A in the different segments of small or large intestine. However, chronic ethanol treatment with a 5% fat diet significantly ($P<0.05$) decreased expression of CYP 3A (lower band) in the jejunum and ethanol treatment with a 40% fat diet produced a significant decrease in CYP 3A1 (upper band) expression in duodenum. This correlated with a significant decrease ($P<0.05$) in testosterone 6 β-hydroxylase activity in duodenal microsomes. Thus, it appears the combination of dietary fat and ethanol treatment are capable of differentially altering CYP 3A expression in the different regions of the small and large intestine. These results are of clinical significance in relation to the first pass metabolism of drugs such as cyclosporine A. Supported by AA08645 (TMB).

381 EFFECTS OF BENZO(A)PYRENE IN RAT LIVER AND LUNG ON CYTOCHROME P450 ISOZYMES

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There is agreement that the environmental contaminant, benzo(a)pyrene, induces P450 1A1 activity. This induction maybe involved in B(a)P carcinogenicity by converting it to a reactive metabolite. Most previous studies have utilized high concentrations of benzo(a)pyrene to study this induction. Further, there is little information regarding the effects of B(a)P on other mixed function oxidase isozymes. The purpose of this investigation was to determine the effects of lower single and multiple doses of B(a)P on mixed function oxidase activity in the rat lung and liver. A one day exposure of 1mg/kg of B(a)P resulted in an increase in both ethoxyresorufin dealkylase activity (CYP1A1) and benzyloxyresorufin dealkylase activity (CYP2B1) in the liver 24 hours after exposure, but returned to control 48 hrs after the exposure. Exposure to 1 mg/kg of B(a)P for 3 days did not alter CYP2B1 activity in the liver, but a 3 day dose of 0.1 mg/kg B(a)P decreased CYP2B1 activity in the liver 48 hrs after exposure. An increase of CYP1A1 activity in the liver was observed 24 and 48 hrs after a 3 day exposure to 1mg/kg B(a)P while 1 day of 0.1 mg/kg produced a decrease in CYP1A1 activity in the liver 24 and 48 hrs after exposure. Administration of this lower dosage for 3 days decreased CYP2B1 activity 48 hrs post exposure, but did not alter CYP1A1 activity in the liver. A single dose of 1mg/kg of B(a)P increased N-nitrosodimethylamine activity (CYP2E1) 24 hrs after exposure while multiple doses of 1 mg/kg B(a)P as well as single and multiple doses of 0.1mg/kg of B(a)P did not result in any changes in CYP2E1 activity in the liver. Administration of 1 mg/kg of B(a)P for 1 day and 0.1 mg/kg of B(a)P for 3 days decreased 2-aminoflourene N-hydroxylase (CYP4B1) activity in the liver. CYP2B1 activity was decreased in the lung 48 hrs after a 3 day administration of 1 mg/kg and 0.1 mg/kg. The one day doses of 1 mg/kg and 0.1 mg/kg of B(a)P resulted in a decrease of CYP2B1 activity 24 and 48 hrs after exposure. The results of this study indicate that single and multiple exposures to 1 mg/kg B(a)P cause a short term increase in CYP1A1 activity in the liver which is without effect in the lung while a single dose of 0.1mg/kg of B(a)P results in a sustained decrease of CYP1A1 activity in the liver up to 48 hrs after exposure. B(a)P also causes organ selective changes in other P450 isozymes.

382 EFFECTS OF ETHYL BENZENE INHALATION EXPOSURE ON CYTOCHROME P450 ACTIVITY IN RAT LIVER, LUNG AND NASAL MUCOSA

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Ethyl benzene is an important industrial precursor and solvent, and is also a major component of gasoline. The inhalation route is a significant exposure pathway to this chemical in the workplace. The effect of inhalation exposure (300 ppm, 6 hours) on P450 isozyme activity was examined in liver, lung and nasal mucosa of Sprague-Dawley rats. In the liver, CYP 1A1 activity (indicated by O-dealkylation of ethoxyresorufin) was not significantly changed, while CYP 2B1 activity (indicated by O-dealkylation of benzyloxyresorufin) was significantly (22.9%) increased. The ratio of CYP2B1 activity to CYP 1A1 activity (termed the benzo(a)pyrene (BaP) detoxication/toxication ratio) was likewise significantly increased, indicating a potential for greater production of a less toxic metabolite. The activities of CYP 4B1, (which metabolizes aromatic solvents and carcinogens such as 2-aminofluorene), CYP 1A2 (indicated by O-dealkylation of methoxyresorufin), and CYP 2E1 (which metabolizes several compounds including nitrosodimethylamine) were not significantly altered by ethyl benzene exposure. In the lung, no change was seen in CYP 1A1 activity, while CYP 2B1 activity was significantly decreased (51.3%), as was the BaP detoxication/toxication ratio. A decline in this ratio could indicate greater production of the more toxic metabolite. No effect was seen in CYP 1A2 activity. The activity of CYP 4B1 was significantly decreased in the lung (64.9%), while CYP 2E1 activity significantly increased (33.5%). In nasal mucosa, in contrast to the lung, there was no significant change in CYP 2B1 activity and a significant decrease in CYP 1A1 activity, resulting in a significant increase in the BaP detoxication/toxication ratio and indicating a shift towards detoxication. Significant decreases in CYP 4B1 (28.9%) and CYP 1A2 (83.8%) activity were seen, while CYP 2E1 activity increased (21.2%). The alterations in isozyme activity observed in this study are both enzyme and organ selective, and can be used to predict the metabolic fate of other xenobiotics upon subsequent or co-exposure.

383 POTENTIATION OF CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY BY ALKYL SULFIDES: THE ROLE OF CYP2E1 EXPRESSION

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The modulation of CCl_4-induced hepatotoxicity in response to alkyl sulfides and alkyl ethers including allyl disulfide (ADS), allyl sulfide (AS), allyl ether (AE), n-propyl disulfide (PDS), n-propyl sulfide (PS), n-propyl ether (PE) and n-butyl sulfide (BS) was studied. Whereas pretreatment of rats with either ADS or AS (50 mg/kg, 7d) blocked CCl_4-induced hepatotoxicity by 91% and 56%, respectively, AE, PDS, PS, PE or BS treatment enhanced CCl_4-induced hepatotoxicity by 52%, 55%, 238%, 25% or 86%, respectively. The molecular basis for the enhanced hepatotoxicity was further studied using PS. Injection of $GdCl_3$ to PS-pretreated rats failed to block the potentiated CCl_4 toxicity, whereas $GdCl_3$ completely prevented vitamin A-enhanced elevation of plasma alanine aminotransferase (ALT) activity. AS treatment completely blocked PS-potentiated CCl_4 intoxication. Concomitant treatment of animals with both PS and vitamin A followed by a CCl_4 insult resulted in super-potentiation of CCl_4-induced hepatotoxicity, suggesting that the mechanism of PS-enhanced hepatotoxicity differs from that caused by vitamin A. Animals pretreated with either pyridine or phenobarbital (50 mg/kg, 7d) followed by an insult with a subtoxic dose of CCl_4 resulted in an ~ 18-fold increase in ALT activity, as compared to CCl_4 alone, implying that CYP2E1 and CYP2B expression may be strongly associated with the potentiated liver toxicity. CYP2E1 expression appeared to be highly associated with the alkyl sulfide- or alkyl ether-modulated hepatotoxicity ($r = 0.92$), as evidenced by both immunoblot analyses and CYP2E1-catalyzed metabolic activity. These results demonstrated that ADS and AS have distinct capability of blocking CCl_4-induced hepatotoxicity whereas saturated alkyl sulfides or alkyl ethers rather potentiated CCl_4-induced hepatotoxicity and that the underlying mechanism is associated with CYP2E1 expression. (Supported by KOSEF 951-0710-001-02)

384 POSSIBLE INDUCTION OF DRUG AND STEROID HORMONE METABOLIZING ENZYME ACTIVITIES THROUGH PCBS EXPOSURE IN WILD POPULATION OF COMMON RAVEN

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This study is about wildlife toxicology of common raven (*Corvus corax*) in Japan. Totally forty ravens were collected from different locations. Residue levels of PCBs including toxic planar isomers were determined in pectoral muscles of these birds. *In vitro* biochemical assays were performed using liver microsomal fraction for drug metabolising enzymes (EROD, AHH and ALDE) and steroid hormone (testosterone, estradiol-17β) metabolising enzymes. Among all the enzymes tested, EROD and testosterone 6β-hydroxylase activities correlated with residue levels of PCBs in wild raven. Some ravens were subjected to dose test of PCBs, 3-methylcholanthrene and phenobarbital. The results showed that estradiol 4-hydroxylase activity was highly induced by PCBs compared to 2-hydroxylase activity. Since the metabolite produced by estradiol 4-hydroxylase, namely 4-catechol estrogen is reported to be an endogenous toxicant in experimental animals, the induction of this enzyme may cause a part of estrogen induced toxicities (*e.g.*, carcinogenicity, covalent binding to macromolecule). Western blots using purified monoclonal antibody against rat P-450 was also performed in wild raven tissues. These results showed that species specific P-450 was possibly induced by PCBs in common raven, and suggested a presence of toxic mechanism of PCBs through endocrine system in wild birds.

385 CYTOKINE REGULATION OF CYP3A2 EXPRESSION IN RATS TREATED WITH LPS

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LPS is a potent inducer of the acute phase response resulting in the release of cytokines such as TNF, IFNα, IL-1, and IL-6. Cytokines can have profound effects on a host of liver proteins, including cytochrome P450 monooxygenases. The CYP3A enzymes metabolize a wide variety of drugs and xenobiotics. Evidence exists for the depression of CYP3A activity, protein, and mRNA, after injection of LPS. The objective of our research is to further define the molecular mechanism(s) by which cytokines may regulate CYP3A2 expression after LPS treatment. Rats were injected i.p. with 2.0 mg/kg LPS

and sacrificed 4 hours later. Nuclear protein was isolated from liver tissue, and binding to various radiolabeled oligonucleotides was analyzed by the electrophoretic mobility shift assay. Binding of nuclear proteins to an NF-IL6 oligonucleotide, consisting of sequences found at -2918 in the CYP3A2 gene, decreased with LPS treatment. In contrast, binding of nuclear proteins to NFkB and AP-1 consensus elements was increased with LPS treatment. The results of our studies indicate potential regulatory roles for NF-IL6, NFkB, and AP-1, in CYP3A2 gene expression. **(A.L.R. supported by NIEHS Training Grant 5 T32ES07266.)**

386 IDENTIFICATION AND CHARACTERIZATION OF CYTOCHROME P450 ISOZYMES IN RAT NASAL EPITHELIUM MICROSOMES

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Since the nose is a major point of entry for xenobiotics, drug metabolizing enzymes in the nasal epithelium are of great importance to understanding toxic effects on this tissue. In this paper, antibodies specific for individual cytochrome P450 isozymes were used to identify the presence of cytochrome P450 isozymes in rat nasal epithelium microsomes. The contribution of individual isozyme in the metabolism of testosterone by nasal epithelium microsomes was also studied. Using western blot analysis, cytochrome P450 1A2-, 2A1-, 2B1-, 2E1-, 3A-, and 4A-related proteins were detected in nasal epithelium microsomes. These six P450 isozymes were found to have the same mobility on SDS-PAGE as their homologues in the liver. Incubation of testosterone with nasal epithelium microsomes yielded 15α-, 15β-, 1β-, and 2β-OH testosterone at much higher rates than rat liver microsomes. In addition, 2α-, 6α-, 6β-, 7α-, 16α-, 16β-, 19-OH testosterone and androstenedione were also formed, however at lower rates. Contrary to liver microsomes, there was no gender-related difference in the metabolism of testosterone by nasal epithelium microsomes. Antibodies against P450 2A1 were able to inhibit the production of 7α-, 1β-, 15α-, 15β-, and 2β-OH testosterone by 90%. In addition, antibodies against P450 3A and 2B1 inhibited 6β-, and 16β-hydroxylation of testosterone, respectively. Treatment of rats with isoniazid was found to induce P450 2E1; β-naphtoflavone induced 1A1 and 1A2; isosafrole induced 1A2; and dexamethasone produced a slight induction of 2A1-related protein. Treatment of rats with isosafrole or acetone was found to decrease P450 3A-related protein. In conclusion, rat nasal epithelium contained several P450 isozymes which differed from those found in the liver in terms of specificity for testosterone hydroxylation and regulation by xenobiotics.

387 INSULIN-DEPENDENT REGULATION OF CYP2E1 AND 2B EXPRESSION IN PRIMARY CULTURED RAT HEPATOCYTES

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Although hyperketonemia and/or altered growth hormone secretion caused by diabetes have been implicated in enhanced CYP2E1 and 2B expression *in vivo*, the effect of insulin itself, in the absence of associated metabolic/hormonal alterations, on hepatic CYP2E1 and 2B expression remains unknown. The effect of insulin on basal and xenobiotic-enhanced CYP2E1 and 2B mRNA and protein expression in primary cultured rat hepatocytes was examined. Primary rat hepatocytes maintained in culture in the absence of insulin for 48, 72, and 96 hr increased basal CYP2E1 mRNA levels 5-, 11-, and 4-fold, respectively, while CYP2B mRNA levels were increased by only 1.6-fold, compared to cells cultured in the presence of 1 μM insulin. Basal CYP2E1 and 2B protein levels were increased 5.5- and 3.5-fold, respectively, in cells cultured in the absence of insulin compared to those cultured in the presence of 1 μM insulin for 96 hr. The addition of 1 μM insulin to cultured hepatocytes for the final 24 or 48 hr of the 96 hr culture period decreased CYP2E1 and 2B mRNA levels by 75–80% and 35– 70%, respectively, relative to the levels present in hepatocytes cultured in the absence of insulin for the 96 hr duration of culture. Decreasing the concentration of insulin below 10 nM (1 nM, 0.1 nM, no insulin) resulted in a 4-, 7-, and 11-fold increase, respectively, in CYP2E1 mRNA levels, but did not affect basal CYP2B mRNA expression. Decreasing the concentration of insulin enhanced the induction of CYP2E1 protein by acetone or pyridine without affecting existing CYP2E1 mRNA levels. CYP2B mRNA and protein induction by acetone, pyridine or phenobarbital was suppressed by decreasing the concentration of insulin. Thus, insulin differentially affects basal and xenobiotic-enhanced expression of CYP2E1 and 2B in primary cultured rat hepatocytes. Supported by NIH Grant ES03656.

388 HYPEROXIA PREFERENTIALLY INDUCES CYTOCHROME P4501A1 ACTIVITY IN RAT LIVER

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Recent studies have suggested an important role for cytochrome P450 enzymes in oxygen-induced tissue injury. In the present study, adult male Sprague-Dawley rats were exposed to hyperoxia (>95% O_2) for 48 or 60 h and liver microsomal ethoxyresorufin O-deethylase (EROD) (CYP1A1) and methoxyresorufin O-demethylase (MROD) (CYP1A2) enzyme activities were determined. In addition, glutathione peroxidase (GP) activities towards cumene hydroperoxide were measured in the cytosols. At 48 h, the EROD and MROD activities were elevated in the hyperoxia-exposed animals over the corresponding activities in air-breathing controls by 7.6-fold and 3.3-fold, respectively. At 60 h, EROD and MROD activities in the hyperoxic animals declined dramatically, to control levels. GP activities were not altered significantly in the hyperoxia-exposed animals at either time point. The results indicate that 48 h of hyperoxia induces CYP1A1 to a greater extent than CYP1A2, in analogy to induction by the potent carcinogen 3-methylcholanthrene. The reduction of cumene hydroperoxide is catalyzed preferentially by glutathione S-transferase Ya subunit, an enzyme encoded by the Ah gene battery. The lack of induction by hyperoxia of this GP activity implies the involvement of alternate mechanisms of induction of CYP1A1/1A2, in contrast with recent proposals that induction CYP1A1/1A2 by hyperoxia may occur *via* an Ah receptor-mediated mechanism. The dramatic decline of CYP1A1/1A2 enzyme activities between 48 and 60 h of O_2 indicates a surprisingly selective alteration, which in turn suggests that these enzymes may be particularly active sources of reactive oxygen species resulting from prolonged hyperoxic exposure. Supported in part by NIH GM44263 and HD27823.

389 EFFECTS OF THE ANTIESTROGEN TAMOXIFEN ON RAT HEPATIC PHASE-II ENZYME EXPRESSION

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The phenobarbital-like properties of tamoxifen (TAM) suggest that the drug may have significant effects on phase-II xenobiotic metabolizing enzyme expression. In order to investigate this, tamoxifen (TAM) was administered to F344 rats and hepatic glutathione-S-transferase (GST), UDP-glucuronosyltransferase (UGT), and sulfotransferase (ST) expression were evaluated. Some of the effects, including dramatic suppression of selected GST enzymes and activity, were observed at a dose in rats which is directly equivalent, on a mg/kg b.w. basis, to the doses used for breast cancer treatment. Interestingly, many of the observed responses are not consistent with the previously described phenobarbital-like properties of TAM, and could be the result of the partial agonist/antagonist activity of TAM at the estrogen receptor. TAM suppressed GST Yal mRNA and protein expression approximately 50% in both males and females. GST Ya2 mRNA and protein expression was suppressed 50% in females yet induced 100% in males. GST Yc expression was induced 40% in males. GST conjugation activity towards ▲ 5 Androstene-3, 17-dione, a substrate which is preferentially metabolized by GST Yal, was suppressed 40% in female rats treated with 0.5 mg/kg TAM, consistent with our HPLC findings. TAM also produced a dose-dependent increase in UGT2B1 mRNA, a phenobarbital-inducible enzyme, in both sexes. In addition, mRNA levels for ST2A1 and ST2A2, two female-specific genes, were weakly suppressed in females and induced in males. Also, ST1C1, an enzyme possibly involved in the excretion of genotoxic TAM metabolites, was weakly suppressed in males. Overall, these results demonstrate TAM-induced effects on phase-II enzyme expression which could be important for the metabolism and disposition of the drug.

390 SKF 525-A INDUCES COCAINE N-DEMETHYLASE, ETHOXYRESORUFIN O-DEETHYLASE AND PENTOXYRESORUFIN O-DEALKYLASE ACTIVITIES BY INDUCTION OF CYTOCHROME P450 2B IN FEMALE B6C3F1 MICE

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Previous studies showed that SKF 525-A inhibited cocaine N-demethylase activity, which has been proposed to be catalyzed by P450 2B1 in rats, in liver S-9 fractions from female B6C3F1 mice. We have speculated that cocaine-induced immunosuppression is mediated by metabolites of cocaine. However, our initial study showed that the antibody response to sheep red blood cells was significantly suppressed by cocaine when mice were co-administered with SKF 525-A. An increase in formaldehyde generation was subsequently shown following the incubation of cocaine with S-9 fractions prepared from SKF 525-A-treated mice, indicating the possibility of P450 induction. Therefore, the inductive effects of SKF 525-A on P450 enzyme activities and proteins were investigated in female B6C3F1 mice to elucidate the effect of SKF 525-A on cocaine-induced immunosuppression. When SKF 525-A was administered at 10, 20 or 40 mg/kg/day intraperitoneally for 7 consecutive days, both ethoxyresorufin O-deethylase and pentoxyresorufin O-dealkylase activities were induced dose-dependently. Furthermore, the induction of enzymatic activity was time-dependent. Meanwhile, when the type of isozyme induced by SKF 525-A was analyzed by Western immunoblotting with mono-specific anti-P450 1A (MC 1–7–1) and anti-P450 2B (PB 2–66–3) antibodies, only the P450 2B appeared to be induced. From *in vitro* inhibition studies with monoclonal antibodies, it was confirmed that the induced activity of ethoxyresorufin O-deethylase by SKF 525-A was due to the increased level of P450 2B proteins. Our present results provide an explanation for the potentiation of cocaine-induced immunosuppression by repeated exposure to SKF 525-A that was seen in our preliminary study. Our results also indicate that ethoxyresorufin O-deethylase, which has been known as a very selective substrate for P450 1A1, may also be catalyzed by P450 2B. (Supported by the NIH grant DA 08161 and by the training grant ES 07087).

391 DISTRIBUTION OF AH RECEPTOR AND CYP1A1 IN MURINE LIVER

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The hepatic distribution of the Ah receptor (AhR) was investigated in C57BL/6J and DBA/2J mice. Proteins of 95 and 104 kDa were detected in immunoblots of cytosol from C57BL/6J and DBA/2J mice, respectively, while a band of 93 kDa was detected in nuclear extracts. The AhR and CYP1A1 were co-localized immunohistochemically in adjacent liver sections. Staining for the AhR was localized mainly in the cytoplasm of centrilobular hepatocytes, whereas the staining in periportal hepatocytes was localized in the nuclei. An additional subset of hepatocytes contained moderative cytoplasmic and low nuclear staining. Staining for CYP1A1 predominated in centrilobular hepatocytes and was found exclusively in the cytoplasm; staining was negligible in periportal hepatocytes. This pattern of distribution was identical for the AhR and CYP1A1 in adjacent liver sections, and was coincidental in individual hepatocytes in extent of immunoreactivity. The distribution of both proteins was similar in control and 3-methylcholanthrene-treated C57BL/6J and DBA/2J mice. These results demonstrated heterogeneity in the distribution of the AhR and CYP1A1, and further that the AhR is localized in both the cytoplasm and nucleus of the hepatocyte. Funded by MRC of Canada.

392 INVESTIGATION OF THE INDUCTION OF 7-PENTOXYRESORUFIN O-DEALKYLATION (PROD) IN MICE TREATED WITH CYP1A ENZYME INDUCERS

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Induction of rat CYP2B by phenobarbital results in a marked increase (> 20 fold) in liver microsomal PROD activity, whereas induction of CYP1A by β-naphthoflavone, 3-methylcholanthrene (3-MC) or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) results in a marked increase (> 20 fold) in liver microsomal EROD activity. Measurement of EROD/PROD activity, therefore, provides an effective method for discriminating between induction of CYP1A and CYP2B in rats. However, treatment of B6C3F1 and CD-1 mice with TCDD increases PROD activity up to 7-fold, which approaches the magnitude of PROD induction by phenobarbital (up to 12-fold). Liver microsomes from mice treated with TCDD or 3-MC contained undetectable levels of immunoreactive CYP2B proteins, suggesting that the ability of these agents to induce PROD is not because they induce mouse CYP2B enzymes. Polyclonal and monoclonal antibodies against CYP1A1 or CYP1A2 markedly inhibited EROD activity (up to 70%) in liver microsomes from TCDD-treated mice. Under similar conditions, these antibodies did not inhibit PROD activity, suggesting that mouse CYP1A1 and CYP1A2 do not catalyze PROD. Kinetic analysis of PROD activity in liver microsomes from B6C3F1 and CD-1 mice revealed that TCDD-treated mice have a kinetically distinct enzyme (K_m = 0.3–0.4 μM, V_{max} = 800–1200 pmol/mg/min) from that present in phenobarbital-treated mice (K_m = 3.1–3.3 μM, V_{max} = 300–320 pmol/mg/min). This kinetically distinct enzyme in TCDD-treated mice catalyzes PROD but does not appear to be a CYP1A or CYP2B enzyme. Supported by NIH grant ES03765.

393 A MONOCLONAL ANTIBODY AGAINST RAT CYP2C7 RECOGNIZES HUMAN CYP2C8, CYP2C19 AND CYP2A6

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Purified CYP2C7 from male Sprague Dawley rats was used as an immunogen in mice for the production of a monoclonal antibody. The antibody was used to probe a bank of human liver microsomes by western immunoblotting, and three electrophoretically distinct proteins of varying intensity were identified. The antibody also was used to probe a panel of cDNA-expressed human P450 enzymes. The antibody recognized two members of the human CYP2C subfamily, namely CYP2C8 and CYP2C19, but failed to recognize CYP2C9 or CYP2C18. The antibody also failed to recognize CYP1A1, 1A2, 2B6, 3A4, 2D6 and 2E1, but did recognize CYP2A6. Of the three proteins recognized in human liver microsomes, the upper band co-migrated with CYP2C8, the middle band co-migrated with CYP2C19, and the lower band co-migrated with CYP2A6. The sample-to-sample variation in the levels of the lower band correlated with coumarin 7-hydroxylase activity, which provides additional evidence that this immunoreactive protein is CYP2A6. Similar experiments are underway to determine if the sample-to-sample variations in the middle and upper immunoreactive proteins correlate with *S*-mephenytoin 4'- hydroxylase and taxol 6-hydroxylase activity, respectively. Analysis of the primary structure of the human P450 enzymes revealed an amino acid sequence that is present in CYP2C8, CYP2C9 and CYP2C19 but absent from the other human P450 enzymes, including CYP2C9 and CYP2C18. This peptide (Asn-Ile-Glu-Asn-Leu-Val-Gly-Thr) is being synthesized to evaluate the identity of the epitope recognized by the monoclonal antibody. Supported by NIH grants ES03765 and ES07079.

394 ETHANOL-ENHANCED COCAINE HEPATOTOXICITY IN MICE: EXAMINATION OF THE ROLE OF NORCOCAETHYLENE

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In the presence of ethanol, cocaine (COC) is metabolized to cocaethylene (CE), which has been shown in mice to have an hepatotoxic potency similar to that of COC. The shift in metabolism of COC by ethanol from formation of non-toxic metabolites (chiefly, benzoylecgonine) to CE has been postulated to contribute, in part, to the increase in COC hepatotoxicity produced by ethanol cotreatment. In the present study, the N-demethylated metabolite of CE, norcocaethylene (NCE), was found in concentrations ≥ CE in the livers of mice treated with COC + ethanol. The rapid formation of NCE from CE was confirmed in experiments in which mice were administered CE directly. NCE concentrations were 3–4 times higher than those of the parent compound, CE, at all time points, including the earliest time point (15 min) after the dose. The apparent half-life for elimination of NCE was longer than that of CE. The hepatotoxic potency of NCE in mice was compared with CE, COC, and the N-demethylated metabolite of COC, norcocaine (NC). On a molar basis, NCE was more potently hepatotoxic than COC, NC, or CE, as determined by serum aminotransferase activities. The hepatotoxicity of NCE was confirmed by histopathology. These studies suggest that the hepatotoxicity of CE is mediated through its N-oxidative metabolism to NCE, and that this metabolic pathway may contribute significantly to ethanol-enhanced cocaine hepatotoxicity. [Supported by DA06601 and DA05585]

395 MINIMAL EFFECT OF ACUTE EXPERIMENTAL HEPATITIS INDUCED BY LIPOPOLYSACCHARIDE/D-GALACTOSAMINE ON BIOTRANSFORMATION IN RATS

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Bacterial endotoxins induce a broad spectrum of toxic reactions. When administered with D-galactosamine, lipopolysaccharide endotoxins produce a good experimental animal model for studying the pathophysiology of hepatitis. This galactosamine plus endotoxin model has been widely used, but few studies have focused on the acute effect of this fixed combination of two chemicals on hepatic and extrahepatic biotransformation. Therefore, either 2 or 4 hr after a single dose (300 mg/kg galactosamine plus 30 μg/kg lipopolysaccharide) was administered intraperitoneally, serum, liver, kidney, intestine and spleen were collected. Serum enzymes (alanine and aspartate aminotransferases, sorbitol dehydrogenase and γ-glutamyltranspeptidase) were dramatically elevated 2 and 4 hr after treatment. Cytochrome P-450 monooxygenase activity toward benzo(a)pyrene was increased in kidney 4 hr after treatment, whereas dealkylation of 7-methoxycoumarin or 7-ethoxyresorufin was unchanged in any tissue at either time point. An increase in UDP-glucuronosyltransferase activity toward 4-methoxycoumarin and 4-hydroxybiphenyl was noted in intestine. Conjugation of 1-chloro-2, 4-dinitrobenzene with glutathione was increased in intestine and spleen 2 hr after treatment. γ-Glutamyltranspeptidase activity was unaltered in all tissues studied. Oxidized and reduced glutathione concentrations were significantly increased by different amounts depending on which organs were studied 2 or 4 hr after treatment. These results indicate that galactosamine/lipopolysaccharide induces liver injury without having major effects on hepatic or extrahepatic biotransformation.

396 THE PROTECTION AGAINST CARBON TETRACHLORIDE (CCl_4)-INDUCED LIVER NECROSIS IN MICE BY NATURALLY OCCURRING METHYLENEDIOXYBENZENES (MDBS)

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The cytoprotective effects and mechanisms of naturally occurring MDBs towards CCl_4-induced liver necrosis in mice were investigated. MDBs were given intraperitoneally prior to CCl_4 administration. Liver microsomes were also used to study the effects of MDBs on CCl_4-induced *in vitro* lipid peroxidation and on cytochrome P450 isozyme activities. Isosafrole, safrole, dihydrosafrole and benzodioxole at doses of 10 mg/kg given 1 hr before the hepatotoxin significantly prevented the increase in plasma transaminase levels and histopathological changes associated with CCl_4-induced liver necrosis. However, piperonyl butoxide (PBO), sesamol and other structure related alkenylbenzenes did not prevent CCl_4 hepatotoxicity even at doses of 200 mg/kg. Isosafrole was not effective if administered even 10 min. after the toxin. Liver microsomes isolated from 1 hr isosafrole-pretreated mice showed a marked decrease in CCl_4-induced *in vitro* lipid peroxidation as well as CYP2E1 catalyzed *p*-nitrophenol hydroxylation. The protection by MDBs was mainly attributed to the inhibition of reductive bioactivation of CCl_4, through formation of metabolic intermediate complexes between reactive MDBs metabolites and ferrocytochrome P450. Isosafrole and safrole were found to be potent *in vitro* inhibitors of CYP2B1 or CYP2E1 dependent metabolism, whereas PBO did not inhibit CYP2E1 activity, suggesting that inhibition of cytochrome P450 isozyme activities by isosafrole may contribute to its hepatoprotective effects by preventing the bioactivation of CCl_4. (Supported by Natural Sciences and Engineering Research Council grants)

397 HEAT SHOCK PROTEIN INDUCTION IN MURINE LIVER AFTER ACUTE TREATMENT WITH COCAINE

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The effect of cocaine on heat shock protein (hsp) induction in murine liver was examined using Western blotting and immunohistochemistry. A single dose of cocaine (50 mg/kg, i.p.) was administered to naive, phenobarbital (PB)- induced or β-naphthoflavone (βNF)-induced mice, and the level of hsps in the liver analyzed 3, 6, and 24 hours after the cocaine dose. As measured by Western blotting, hsp25 and hsp701 were induced at each of the time points, though no apparent induction was observed for other hsps (hsp60, hsc70, or hsp90). Pretreatment of mice with the cytochrome P-450 inhibitor SKF-525A eliminated both cocaine hepatotoxicity and induction of hsp25 and hsp701. Immunohistochemical localization of hsp25 and hsp701 in the liver showed that induction of both hsps was restricted to cells with altered morphology. As has been observed previously, hepatic enzyme induction with PB or βNF shifted the location of the necrotic lesion within the lobule from zone 2, as observed in naive mice of this strain, toward zone 1 (PB) or zone 3 (βNF). Localization of induction of hsp25 and hsp701 was found to shift within the lobule in parallel with the necrotic lesion in these animals. Immunostaining of cocaine reactive metabolites bound to proteins was superimposable on the areas with hsp induction and cells with altered morphology. Our observations indicate a strong spatial correlation within the lobule between cocaine reactive metabolite formation, hsp25 and hsp701 induction, and cytotoxicity (necrosis). Temporally, hsp25 and hsp701 induction accompanied the rapid development of altered cell morphology following cocaine administration in vivo. [Supported by ES-07213].

398 ROLE OF UNCOUPLING IN INDUCTION OF THE MITOCHONDRIAL PERMEABILITY TRANSITION BY SALICYLATE AND VALPROIC ACID

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Recently, we showed that salicylate and other drugs implicated in Reye's syndrome and Reye's-related disorders induce the mitochondrial permeability transition (MPT) (AASLD Single Topic Symposium on Oxidative Stress and Liver Disease, Airlie, VA, June 22–25, 1995). Since salicylate is a weak uncoupler, and because opening of a voltage-gated channel causes onset of the MPT, our aim was to determine whether drug-induced uncoupling was the mechanism underlying induction of the MPT. To monitor mitochondrial $\Delta\Psi$, isolated rat liver mitochondria (0.3 mg protein/ml) were incubated in Mg^{2+}-containing buffer with 0.6 μM rhodamine 123 at 30 °C. Rhodamine 123 distributes electrophoretically into the matrix of polarized mitochondria causing a red spectral shift measured by the 516–495 nm absorbance difference. Succinate (5 mM) produced rapid mitochondrial polarization. Salicylate (75 – 300 μM) caused modest dose-dependent depolarization with 300 μM collapsing $\Delta\Psi$ only 20%. This was comparable to the uncoupling of 5 nM FCCP. In a standard swelling assay for the MPT in Mg^{2+}-free medium, as little as 37.5 μM salicylate induced the MPT, whereas 5 nM FCCP had no effect. The anticonvulsant, valproic acid, also causes Reye's-like toxicity. Valproic acid (37.5 – 300 μM) induced the MPT at concentrations having virtually no effect on $\Delta\Psi$. These data indicate that induction of the MPT by agents implicated in Reye's-like syndromes is not the consequence of direct mitochondrial uncoupling.

399 BILE ACID SECRETION BY RAT HEPATOCYTES CULTURED IN A COLLAGEN GEL SANDWICH CONFIGURATION

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Rat hepatocytes cultured on collagen coated dishes are often used as a model for the study of bile acid synthesis and metabolism. However, hepatocytes cultured in this manner lose most liver specific functions within a week and assume a dedifferentiated condition similar to fetal hepatocytes. Furthermore, bile acid synthesis, metabolism, and transport are impaired. In contrast, hepatocytes cultured between two layers of collagen gel (a collagen gel "sandwich" configuration-CGS) assume a normal appearing phenotype and maintain liver specific functions, including bile acid secretion, for at least 6 weeks. We have further characterized this sandwich system for its suitability for bile acid studies, comparing it to cells cultured for two weeks either on collagen coated dishes, a single layer of collagen gel, or a collagen gel/matrigel sandwich. In contrast to hepatocytes cultured on collagen coated dishes, the CGS and collagen gel/matrigel systems maintained viability for the entire two weeks. Total bile acid secretion was highest in the CGS system (~600 pmoles/mg protein/hour) followed by the collagen gel/matrigel system. These systems secreted bile acids at significantly higher rates than either the single collagen gel or collagen coated cultures. In the presence of serum, the major individual bile acids secreted by the CGS system were deoxymuricholic, tauro-β-muricholic, taurohydeoxycholic, and taurocholic acid. In addition, by using a ribonuclease protection assay, we were able to demonstrate maintenance of expression of the cholesterol 7α-hydroxylase gene, the rate limiting enzyme in the bile acid synthesis pathway. Therefore, these experiments confirm the suitability of the CGS for the study of bile acid metabolism and synthesis.

400 COCAETHYLENE FORMATION AND N-OXIDATIVE METABOLISM MEDIATE ETHANOL POTENTIATION OF COCAINE HEPATOTOXICITY

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Cocaine hepatotoxicity is potentiated by acute ethanol exposure. Cocaethylene, a unique metabolite of cocaine, is produced by cultured rat hepatocytes when simultaneously exposed to cocaine and ethanol. Like cocaine, cocaethylene is cytotoxic to cultured rat hepatocytes and undergoes N-demethylation and is presumed to undergo subsequent N-oxidation. To assess the contribution of cocaethylene formation and metabolism to ethanol potentiation, the present study examined the effects of acute ethanol and methanol on cocaine cytotoxicity and metabolism in phenobarbital-induced cultured hepatocytes from Wistar rats. As previously observed, cocaine (50–400 μM) produced time- and concentration-dependent release of lactate dehydrogenase (LDH) and depletion of reduced glutathione (GSH). Simultaneous exposure to ethanol (20–60 mM) potentiated these effects. By contrast, simultaneous exposure to methanol (20–60 mM) was without effect. Exposure to either ethanol or methanol alone produced no significant effects. In the presence of 200 μM cocaine, potentiation of LDH release (59 vs 81% of total) and GSH depletion (78 vs 65% of total) by 60 mM ethanol occurred at 6 and 4 hr, respectively. Again, concurrent methanol exposure and ethanol or methanol alone was without effects. Ethanol-mediated potentiation of cocaine cytotoxicity was preceded by the appearance of cocaethylene and norcocaethylene at 1.5 and 3 hr, respectively, as determined by GC-MS. Neither metabolite was identified following exposure to cocaine and methanol. The timing of cocaethylene and norcocaethylene formation relative to cocaine toxicity supports a role for these metabolites in the potentiating effects of ethanol. The absence of these metabolites and the lack to potentiation of cocaine toxicity by methanol further supports this conclusion. (Supported by DA 08215)

401 STIMULATION OF O_2 UPTAKE DUE TO PGE_2 IS OXYGEN-DEPENDENT IN PERFUSED RAT LIVER

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Prostaglandin E_2 ($PGE)_2$) stimulates O_2 consumption by isolated parenchymal cells. Since O_2 plays an important role in the regulation of mitochondrial O_2 metabolism, the aim of this study was to determine if the effect of PGE_2 on hepatic O_2 uptake was affected by O_2 tension. Livers from female Sprague-Dawley rats were perfused at normal or high flow rates (4 or 8 ml/g liver/min) to deliver O_2 to the organ at various rates. During perfusion at normal flow rates, PGE_2 (5 μM) increased O_2 uptake by about 50 μmol/g/h. When livers were perfused at high flow rates, however, PGE_2 increased O_2 uptake by nearly 100 μmol/g/h. Thus, increases in O_2 uptake were directly proportional to O_2 delivery. Further, Infusion of PGE_2 into livers perfused at normal flow rates increased state 3 rates of O_2 uptake of subsequently isolated mitochondria by about 25%. Rates were increased much more by prior PGE_2 infusion in mitochondria isolated from livers perfused at high flow rates. e.g. state 3 rates of O_2 uptake were increased by about 70% by high flow rates. PGE_2 stimulated O_2 uptake two-fold more in mitochondria isolated from livers perfused at high than at normal flow rates. Taken together, These data indicate that the effect of PGE_2 on mitochondrial respiration is O_2-dependent in the perfused liver (AA-09156 and AA-03624).

402 INTESTINAL UDP-GLUCURONOSYLTRANSFERASE ACTIVITIES IN RAT AND RABBIT

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Although the liver is the primary site of xenobiotic metabolism, other tissues contribute and can significantly alter bioavailability. This study investigated glucuronidation in rat and rabbit small intestine microsomes and compared it to glucuronidation in liver microsomes. UDP-glucuronosyltransferase (UGT) activities against 4-methylumbelliferone (MU), 4-nitrophenol (NP), 1-naphthol (1-N), 2-naphthol (2-N), 4-hydroxy- (4-HB) and 2-hydroxy- (2-HB) biphenyl, phenolphthalein (PHTH), diclofenac, and testosterone were determined using TLC separation of glucuronides generated in incubations performed with ^{14}C-UDPGA. Activities were determined from densitometric quantitation of autoradiographs of the TLC plates and are reported as nmol/mg/min. UGT activities towards MU, NP, 1-N, and 2-N were all between 23-30 in rat liver. In rabbit liver, MU and NP activities were approximately 50% of the rat values whereas 1-N and 2-N were less than 25%. In rabbit small intestinal microsomes UGT activities were 10 for MU, and 4.5 for NP, 1-N, and 2-N. In rat intestinal microsomes, UGT activity towards MU was again the highest among the four substrates, but with an activity of only 3.5. Activities toward NP, 1-N, and 2-N were 3.0, 1.5, and 2.5 respectively. Rat and rabbit liver microsomes possess similar UGT activity toward 4-HB (4.0) but rabbit intestinal activity was 3-fold greater than that of the rat (1.5 vs 0.5). UGT activity toward 2-HB was higher in rat when compared to rabbit liver (2.3 vs 0.7) while the reverse was observed in the small intestine (0.4 vs 0.8). For PHTH, diclofenac, and testosterone, UGT activities were 1.5, 1.3, and 1.6 respectively in rat liver and 1.3, 0.5, and 0.4 in rabbit. In intestinal microsomes, activity with these substrates was absent in rat whereas in rabbit, activities were 1.6, 0.2, and 0.2 respectively. Thus, UGT activities for most aglycones were higher in rat liver microsomes than in rabbit, but the reverse was true for intestinal microsomes suggesting that intestinal UGTs could contribute more to overall glucuronidation with rabbit as the animal model as compared to rat.

403 INHIBITION OF INDUCIBLE NITRIC OXIDE SYNTHASE WITH AMINOGUANIDINE ABROGATES ACETAMINOPHEN-INDUCED HEPATOTOXICITY

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Treatment of male LEH rats (100 g) with acetaminophen (AA, 1 g/kg, i.p., 24 hr) results in centrilobular hepatic necrosis. This was associated with an accumulation of macrophages in centrilobular regions of the liver and increased production of proinflammatory and cytotoxic mediators including tumor necrosis factor-α (TNF-α), platelet activating factor (PAF), superoxide anion and inducible nitric oxide synthase (iNOS) which have been implicated in tissue injury. The macrophage inhibitor, $GdCl_3$ (7 mg/kg, i.v.), blocks AA-induced hepatotoxicity, as well as inflammatory mediator accumulation, demonstrating that these cells and their products contribute to injury. To analyze the role of nitric oxide in tissue injury, we used various inhibitors of nitric oxide synthase. Treatment of rats with N^G-methyl-l-arginine, Nω-nitro-l-arginine methyl ester or aminoguanidine blocked expression of iNOS in the liver. This was correlated with reduced hepatic necrosis and serum ALT and AST levels. Aminoguanidine, which has more specificity for iNOS, was the most effective of the inhibitors. Aminoguanidine treatment also abrogated AA-induced expression of TNF-α and PAF in the livers. These data demonstrate that nitric oxide contributes to AA hepatotoxicity and suggest that this mediator may regulate cytokine production in the liver (NIH GM34310 & The Burroughs Wellcome Fund)

404 PHOSPHOLIPID SECRETION INTO BILE IS MARKEDLY DIMINISHED BY 1, 1-DICHLOROETHYLENE (DCE)

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Background: DCE produces selective injury to the bile canalicular membrane of hepatocytes within 2 hrs. Functional changes include selective impairment of certain pathways of bile formation, eg., organic anion secretion, while bile salt secretion is unchanged. Our working hypothesis is that the canalicular injury results from interactions of reactive DCE-glutathione conjugates with pericanalicular components. Objective: Determine the time-course of DCE effects on the biliary output of phospholipids which is a function mediated by the canalicular membrane mdr2 P-glycoprotein. Experimental Design: Freely moving male Sprague-Dawley rats equipped with a permanent biliary cannula for bile collection and a duodenal cannula for taurocholate infusion were treated with DCE (50 mg/kg po) or mineral oil vehicle. Results: Biliary output of phospholipids decreased to 25% basal by 30 minutes after DCE in rats infused with physiological amounts of bile salt. Since the presence of DCE-glutathione conjugates within the canalicular lumen could inhibit mixed micelle formation, we measured phospholipid output at later times when biliary excretion of DCE metabolites is greatly diminished. Biliary phospholipid output remained at <50% basal levels for up to 10 hrs after DCE. Since phospholipid secretion is a bile salt-dependent function, we examined the effects of low and high levels of bile salts. When taurocholate infusion was increased to 3x physiologic levels at 2 hr after DCE, phospholipid secretion was not restored in DCE treated rats. When rats were first depleted of bile salts by bile collection without taurocholate replacement, subsequent DCE treatment resulted in a complete absence of phospholipid in bile. Conclusion: The sustained decrease in phospholipid output observed after DCE is

consistent with a bile salt-independent effect on the canalicular membrane mdr2 P-glycoprotein. (Supported by NIH DK 34806 and an Amaco Fellowship)

405 PRE-FIBROTIC EVENTS INDUCED BY IRON FROM FERROCENE IN A RODENT MODEL FOR HEPATIC IRON STORAGE DISORDERS

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Iron is an essential trace element. However, its cytotoxic properties become apparent when metabolic disorders in absorption lead to prolonged overload in tissue organs. Although the clinical consequences of iron storage disorders are well known (hepatic fibrosis & cirrhosis), the early biochemical events leading to permanent damage and an adequate animal model for studying them remain unresolved. We have developed a new rodent model for studying iron storage overload, and investigated proposed mechanisms for iron-induced hepatocellular injury. Sprague-Dawley rats were fed iron from ferrocene in a high fat liquid diet (35% fat-derived cal.) for a period of 52 days (Fe). Controls received the diet with out ferrocene (Ctrl). Hepatic non-heme iron was measured in whole tissue and subcellular fractions, and graded histochemically by Perl's staining. Liver injury was assessed by pathology scores, serum ALT activities, and hyroxyproline (HO-Pr.) accumulation. Oxidative stress was evaluated by measurement of malondialdeyde (MDA), retinol, and glutathione (GSH) in whole hepatic tissue and subcellular fractions. The Fe grp. demonstrated a 26-fold increase in total hepatic non-heme iron over Ctl. with 3, 19, and 22-fold increases in cytosolic, microsomal, and mitochondrial fractions respectively. The Fe grp. had severe hepatic siderosis (grade 4+), 2-2.6-fold elevations in MDA content of mitochondria and microsomes, and 2-fold decreases in retinol and cytosolic GSH content. Iron-induced hepatocellular injury was evident from elevated ALT values (2-fold) and HO-Pr. content (5.7-fold) in Fe over Ctrl. Liver pathology scores confirmed injury with the presence of mild-moderate necrosis and spotty inflammation. No fibrosis was observed. These data suggest an early hepatocellular response to iron-induced oxidative stress before the occurrence of fibrotic morphological changes. (NIH/AA09300)

406 DEVELOPMENT AND CHARACTERIZATION OF AN ACUTE MODEL OF TACRINE HEPATOTOXICITY

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Tacrine, a reversible acetylcholinesterase inhibitor, is the only approved drug for treatment of Alzheimer's disease. Reversible hepatotoxicity in about 30% of patients has limited clinical use. The purpose of this study was to develop and characterize an acute model of tacrine hepatotoxicity to study mechanisms of injury. Rats were given tacrine (10–35 mg/kg, i.g.) and sacrificed 24 hours later. There was a dose-dependent increase in serum aspartate aminotransferase (AST) which was significant at 35 mg/kg (117 ± 16 U/l) when compared to controls (39 ± 14 u/l). Liver histology revealed pericentral necrosis and fatty changes in midzonal regions. Livers were perfused with trypan blue (0.5 mM) to stain dead cells. The time for trypan blue to distribute evenly throughout the liver (6.9 ± 1.6 min) was increased by tacrine compared to controls (1.0 ± 0.3 min), illustrating disturbances in the liver microcirculation. Additionally, liver surface O_2 (69.0 ± 4.8 μM) was decreased compared to controls (89.0 ± 3.5 μM), indicative of hypoxia. Since liver microcirculation is regulated by a cholinergic ganglion, we hypothesized that tacrine, by inhibiting acetylcholine breakdown, increases sympathetic activity to the liver leading to vascular constriction, hypoxia and liver injury. The hepatic nerve was severed to block sympathetic activity to the liver. This procedure blocked both the increases in serum AST and the elevated time for trypan blue to distribute evenly. Additionally, the hepatotoxicity could occur during reoxygenation of hypoxic regions as tacrine is cleared, leading to the production of free radicals. When catechin (100 mg/kg, i.p.), a radical scavenger, was given before tacrine, injury was decreased by about 45%. These data suggest that tacrine hepatotoxicity is a classic hypoxia-reoxygenation injury mediated by inhibiting acetylcholinesterase in the celiac ganglion to increase sympathetic activity to the liver. (GM 07040–21)

407 EXERCISE-INDUCED ALTERATIONS IN THE METABOLISM OF METHYLTESTOSTERONE IN PRIMARY RAT HEPATIC CELL CULTURES

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The effects of exercise are known to induce many cellular adaptations in various organs. Among these organs is the liver. Exercise-training has been reported to effect the P-450 and aspartate aminotransferase (AST) enzyme activity levels, blood flow to the liver, and glutathione pools. These alterations in liver function have the potential to effect drug metabolism and/or toxicity. Anabolic-androgenic steroids (AAS) are an increasingly popular drug of abuse among physically trained individuals. Therefore, the purpose of this study was to evaluate the effects of exercise-training on the metabolism of a commonly abused 17 α-alkylated AAS (methyltestosterone) in primary liver cell cultures. Cultures were established from 112–161 day old sedentary (S) and exercise-trained (ET) male Sprague-Dawley rats. Swim-training consisted of 75-min swim sessions (2 per day), 5 days a week, for 8–15 weeks. Cultures from both groups were administered a 100 μM concentration of methyltestosterone. A 100 μL sample was taken at 0, 0.17, 0.33, 0.5, 1, 2, 4, 6, and 9 hr and analyzed on an HPLC. The rate of disappearance of the parent compound (methyltestosterone) was determined and the concentration and half-life ($t_{1/2}$) calculated from S and ET cell cultures. When comparing the concentration of methyltestosterone in the S and ET groups, the methyltestosterone concentration was 37 and 51% higher in the ET group than that of the S group at 6 and 9 hr, respectively. Furthermore, when comparing the rate of disappearance between the two groups, the $t_{1/2}$ in the S group was 4.3 hr while in the ET group, the $t_{1/2}$ was 8.3 hr. Thus, the parent compound, methyltestosterone, remains present for a longer period of time in the exercise-trained liver. With respect to the half-life, these results indicate that exercise-training affects methyltestosterone metabolism in the liver. How this exercise-induced effect on metabolism relates to AAS-induced hepatotoxicity remains to be determined.

408 MECHANISM OF PHOSPHOLIPID ACCUMULATION IN RATS CAUSED BY SK&F 106615, A NOVEL CATIONIC AMPHIPHILIC AZASPIRANE

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Cationic amphiphiles, such as chloroquine or amiodarone, cause accumulation of phospholipid in different tissues in a variety of animal species. The purpose of this work was to (1) determine if treatment of rats with the novel cationic amphiphilic azaspirane, SK&F 106615, caused phospholipid accumulation in liver; and (2) to investigate possible mechanisms underlying phospholipid accumulation. Male Sprague-Dawley rats were treated for 28 days with 10 mg/kg/day of SK&F 106615. Livers were removed at necropsy and portions of the right and left lateral lobes were either quick-frozen or were cut into approximately 1 mm^3 cubes and fixed and processed for electron microscopic analysis. Frozen liver was extracted with chloroform/methanol and phospholipid classes were separated via TLC/FID. Subclasses within phosphatidycholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) were separated and molecular species were quantitated via HPLC. Multilamellar inclusions consistent with accumulation of phospholipid were observed by electron microscopy in hepatocytes and Kupffer cells. Total hepatic phospholipid content and phospholipid class distribution were not altered by SK&F 106615 treatment, but arachidonate-containing species in the plasmalogens within PC and PE, but not PI, were increased significantly. In contrast, diacyl-linked species were not affected. SK&F 106615 also inhibited phospholipase A_2 but not phospholipase C or D activities *in vitro*. The data suggest that treatment of rats for 28 days with SK&F 106615 caused an inhibition of hepatic plasmalogen-selective phospholipase A_2 *in vivo*, with a concomitant accumulation of phospholipids. This accumulation is expected with a cationic amphiphilic compound.

409 THROMBIN IS A CRITICAL AND DISTAL MEDIATOR OF LPS-INDUCED LIVER INJURY IN VIVO

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The intravenous administration of gram-negative bacterial endotoxin (lipopolysaccharide, LPS) to rats results in the accumulation of both platelets and

neutrophils within the liver and in hepatic parenchymal cell injury within 6 hrs. The coagulation system is critical in the pathogenesis of LPS-induced liver injury since pretreatment with warfarin or heparin provides protection. These and other studies point to thrombin as a critical mediator of liver injury in this model. To test further the hypothesis that thrombin is involved in LPS-induced liver injury, the selective thrombin inhibitor, hirudin, was used. The hirudin treatment regimen effectively inhibited thrombin and prevented LPS-induced liver injury, as measured by elevations in plasma alanine aminotransferase activity and histologic evaluation. Hirudin did not alter hepatic platelet accumulation observed after LPS administration. These results suggest that thrombin is involved in the liver injury but does not act by promoting platelet accumulation.

Since the coagulation system is not activated until shortly before the onset of liver injury, we hypothesized that an activated coagulation system may act distally in the chain of inflammatory events that culminates in hepatocellular necrosis. To test this, animals were given heparin or hirudin at various times after the administration of LPS. Treatment with either heparin or hirudin 2.5 hrs after LPS was associated with reduced liver injury from LPS. These results, taken together, suggest that thrombin is a critical and distal mediator in the pathogenesis of LPS-induced liver injury in the rat. (Supported in part by NIH grant ES04139).

410 REPEATED DOSING WITH CLOFIBRATE DECREASES THE TOXICITY OF MODEL HEPATOTOXINS

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Pretreatment of mice with clofibrate (CFB) has been shown to protect against acetaminophen (APAP) hepatotoxicity. To determine if pretreatment with CFB prevents the toxicity of other hepatotoxins, male CD-1 mice received 500 mg CFB/kg, i.p., daily for 10 days. Controls were given corn oil (CO). They were fasted overnight and killed 24 hr after challenge with either 600 mg bromobenzene (BrB)/kg, 0.25ul carbon tetrachloride (CCl_4)/kg or 0.5 ml chloroform ($CHCl_3$)/kg; all in CO vehicle. Liver and kidney injury was assessed by plasma sorbitol dehydrogenase activity (SDH) and blood urea nitrogen (BUN) respectively. Challenge with BrB resulted in 90% mortality in CO pretreated mice (8/9), with no mortalities detected in the CFB group. In CFB/BrB mice, SDH and BUN were 152 ± 95 U/l and 22.8 ± 2.5 mg/dl respectively, indicating that there was neither hepatotoxicity or nephrotoxicity in CFB pretreated, BrB challenged mice. Similarly, CFB pretreatment diminished CCl_4-induced liver injury (SDH in CO/CCl_4 = 4450 ± 534 U/l vs 554 ± 373 U/l in CFB/CCl_4), with no BUN elevation detected in either group. Partial protection against $CHCl_3$ hepatotoxicity was observed in CFB mice (SDH in CO/$CHCl_3$ = 1553 ± 748 U/l vs 563 ± 69 U/l in CFB/$CHCl_3$); however, BUN was significantly elevated in both groups (54.90 ± 0.4 in CO/$CHCl_3$ vs 56 ± 0.6 in CFB/$CHCl_3$), indicating that CFB did not protect against-$CHCl_3$ induced nephrotoxicity. Histopathological examination of liver and kidney sections confirmed these results. This study shows that pretreatment with CFB also protects against other model hepatotoxins besides APAP. (UConn CBTox).

411 LYSOSOMAL SEQUESTRATION AND LAMELLAR BODY FORMATION INDUCED BY A PYRROLOPYRIMIDINE IN VITRO AND IN VIVO

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In preclinical development studies, a pyrrolopyrimidine drug candidate, U-101033, was found to produce toxicity specific for the gallbladder in dogs, monkeys and guinea pigs. Cytoplasmic lamellar inclusion bodies were coincidentally observed in dog gallbladder epithelial cells and macrophages. The present study was designed to determine the cytological distribution of the drug or drug-related material in dogs and also determine the relationship of drug exposure to lamellar bodies in cultured dog gallbladder epithelial cells (DGBE) and primary hepatocytes. Two beagle dogs were dosed orally with ^{14}C-U-101033 at 100 mg/kg (63 μCi/kg) then euthanized at 4 and 48 hr. At necropsy, liver and gallbladder were collected for scintillation counting and autoradiography. Scintillation counting indicated approximately 15-fold more drug related material in liver and gallbladder than in whole blood at 4 hr, and levels remained comparatively high at 48 hr. Autoradiographic studies suggested drug-related materials to be associated with lysosomes in gallbladder epithelial cells and macrophages. In cultured DGBE and monkey hepatocytes (MH), U-101033 was tolerated at concentrations ≤100 μM. At non-cytotoxic concentrations, it induced fluorescent inclusion bodies in DGBE co-incubated with NBD-phosphatidylcholine, a fluorescent marker used for detecting lysosomal phospholipidosis. Inclusions were dose-dependant and reversible. Co-cultures of DGBE and MH treated with U-101033 under various protocols did not reveal any remarkable differences in cytotoxic sensitivity between these cell types, suggesting that metabolism was not a prerequisite for toxicity. Our data suggest that U-101033 is a lysosomotropic drug, with the lysosome as a significant organellar target.

412 INTESTINAL TOXICITY AND CHANGES IN PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) LEVELS IN HAMSTERS INDUCED BY A CAMPTOTHECIN ANALOGUE

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The camptothecin antitumor compound, CPT-11 (irinotecan hydrochloride), is a topoisomerase I inhibitor currently in clinical trials. Though effective against a variety of tumors, the clinical dose has been limited by incidents of intestinal toxicity (diarrhea). In this study, we examined the hamster as a model for CPT-11-induced intestinal toxicity. Female Syrian hamsters were dosed intraperitoneally with CPT-11 at 50 mg/kg/day for ten days and observed through day 20; animals were sacrificed on days 1–5 for histological and biochemical analysis of jejunum, ileum and colon. By day 5, all treated animals had developed diarrhea, and deaths occurred beginning at day 7. Histologically, a time-dependent loss of structural integrity of the mucosal epithelium was observed the jejunum and ileum; villi appeared corrugated and epithelial cells lost their typical columnar morphology. The epithelium of the colon appeared thinned and vacuolated. There was an apparent reduction of crypts and/or a loss of epithelial cells. Using Western immunoblot techniques, a time-dependent elevation in proliferating cell nuclear antigen (PCNA) was detected in jejunum and colon (ileum was not analyzed) as early as day 1. Immunohistochemistry for PCNA showed an increase in the number of labelled epithelial cells and labelling intensity in treated animals, with labelled cells positionally located farther towards the villus tip compared to controls. The increased levels of PCNA and loss of differentiated cell morphology suggest that CPT-11 induced a cell cycle block in S-G_2 with a subsequent loss of physiological (differentiated, G_1) function in hamster intestinal epithelium.

413 INCREASE IN PLASMA ENZYME ACTIVITIES UNDER CONDITIONS OF INCREASED GLUCONEOGENESIS VIA CARBOHYDRATE DEPLETION IN RATS

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Acarbose (A) is a potent inhibitor of intestinal α-glucosidases. This effect offers a new pharmacological approach for oral antidiabetic therapy by preventing postprandial hyperglycemia and hyperinsulinemia in diabetic patients. At high dietary doses an increase in the plasma activity of ASAT and ALAT has been observed in rats. Since these doses did not result in hepatocellular damage we speculated that enhanced gluconeogenesis might be the cause of this increase. To test this hypothesis 10 female SD-rats per group were administered either A at dietary concentrations of 0, 1500 or 4500 ppm or a low carbohydrate (LC) diet with normal protein (NP) or high protein (HP) content for 4 weeks. Food intake and body weights were determined weekly, feces dry weight, excretion of undigested complex carbohydrates UCC (determined as glucose) and ASAT, ALAT, and GIDH activity in plasma were determined at week 4.

Food intake was increased with LC/HP and LC/NP by +19% and +90% resp and in the A groups by +22% and +47% resp. Feces excretion was increased by +160% and +489% in the diet groups and +215% and +326% in the A groups resp. The unpharmacologic high A doses used result in carbohydrate malabsorption. Excretion of UCC was increased from 16.7 mg glucose/g feces (dry weight) in controls, up to 280 mg/g and 460 mg/g in the A groups resp. This carbohydrate malabsorption due to exaggerated pharmacodynamic activity leads to increased gluconeogenesis comparable with the diet low in carbohydrates. Consequently, the 3 enzymes were significantly increased after A. LC-feeding significantly increased ASAT and GIDH more pronounced with NP. Increase was highest with GIDH (up to 28fold at LC/NP). In the absence of histopathological changes these data suggest that the higher plasma enzyme activities are due to increased gluconeogenesis

in the liver from glucogenic aminoacids, whereby enzymes involved in the aminoacid/glucose metabolism can be markedly induced.

414 PERFUSION WITH LOW-Na^+ MEDIUM PROTECTS ISOLATED RAT LIVER AGAINST HYPOXIA

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A recent study has shown that incubation of hepatocytes under hypoxic conditions in a Na^+-free medium reduced hepatocyte swelling and loss of viability (*Carini R et al, BBRC 1995*). Although isolated hepatocytes represent a reliable model for investigating the effects of hypoxia, more complex models are necessary to confirm results obtained by cell suspensions. Livers obtained from male Wistar fasted rats were perfused via the portal vein with KHB (95% O_2 and 5% CO_2). During hypoxia, livers were perfused for 100 min with KHB equilibrated with 95% N_2 and 5% CO_2. In experiments with low Na^+ (25 mM), NaCl was substituted with equimolar choline chloride. LDH in the effluent perfusate and bile flow were measured at time intervals. The perfusion of isolated livers with N_2-saturated KHB caused an increase of LDH beginning at 20 min and reaching a peak at 50 min. When livers were perfused with N_2-saturated buffer containing low Na^+, the increase of LDH release was delayed, beginning at 60 min and slowly increasing in the following min. During liver perfusion with KHB, hypoxia determined a rapid decline of bile flow which ceased after 40 min. On the contrary, when livers were perfused under hypoxic conditions with low-Na^+ KHB, bile flow diminished slowly and continued for the total period of perfusion. This study demonstrates that extracellular Na^+ plays a relevant role in hypoxia-induced (1) alterations of liver functions and (2) liver necrosis.

415 EFFECT OF COLCHICINE AND VINBLASTINE ON THE PIPERONYL BUTOXIDE INDUCED INCREASE IN BILIARY EXCRETION OF α-TOCOPHEROL (α-T), PHOSPHOLIPIDS, AND BILE ACIDS IN THE RAT

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α-Tocopherol (α-T), a lipid soluble chain-breaking antioxidant, is transported in the body by association with lipids. It has been estimated that the liver contains up to 29% of the α-T stores of the body. Piperonyl butoxide (PIP) is a widely used synthetic methylenedioxyphenyl insecticide synergist. In the current study, bile was collected from rats for 1 hour prior to and 5 hours following i.p. injection of 1g/kg PIP. Within 3 hours of PIP administration biliary α-T levels had increased to 132% of pretreatment levels, while α-T levels in control bile dropped to 80% of pretreatment levels. Biliary levels of bile acids and phospholipids also increased during the first three hours after PIP administration. In a second set of experiments, rats were pretreated with either 2.5mg/kg colchicine, 3.3mg/kg or 13.3mg/kg vinblastine; both colchicine and the higher dose of vinblastine prevented the PIP induced increase in biliary excretion of α-T, bile acids, and phospholipids. We conclude that hepatic α-T is a highly mobilizable source of antioxidant for possible rapid enterohepatic distribution and that microtubules play a role in this distribution. Various chemicals may be able to cause a similar depletion of liver α-T. This work was supported by NIEHS grants ES00210 and ES01978.

416 IDENTIFICATION OF FACTORS FROM RAT NEUTROPHILS RESPONSIBLE FOR CYTOTOXICITY TO ISOLATED HEPATOCYTES

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Activated polymorphonuclear neutrophils (PMNs) are cytotoxic to rat hepatic parenchymal cells (HCs) in vitro. This effect can be observed without direct cell-cell contact, since the conditioned medium from PMNs activated with f-met-leu-phe (fmlp) is toxic to HCs. To identify the cytotoxic factor(s) released by activated PMNs, degranulation was induced by fmlp in PMNs pretreated with cytochalasin B. The contents released were subjected to gel filtration on a Sephadex G-100 column, and resulting fractions were tested for cytotoxicity to isolated HCs using release of alanine aminotransferase as a marker for HC injury. Cytotoxicity was associated with fractions containing cathepsin G and elastase and not with other fractions, including those containing myeloperoxidase. The former two enzymes were purified to homogeneity with a carboxymethyl cellulose column. Each of these enzymes demonstrated concentration-dependent cytotoxicity to HCs at concentrations greater than 2 μg/ml. Moreover, they exhibited an additive cytotoxic effect. The effective concentrations for the combined cathepsin G and elastase in the incubation mixture were similar to the concentrations of these enzymes in PMN-conditioned medium that was toxic to HCs. The relative potencies of various protease inhibitors toward cathepsin G or elastase was the same as their abilities to prevent cytotoxicity of these enzymes or of PMN-conditioned medium. These results support the hypothesis that cathepsin G and elastase are important mediators of HC killing produced by activated PMNs in vitro. Supported by ES04911 and ES04139.

417 GADOLINIUM CHLORIDE PROTECTS FROM CARBON TETRACHLORIDE BUT NOT CADMIUM CHLORIDE TOXICITY IN ISOLATED RAT HEPATOCYTES: THE ROLE OF CYTOCHROME P450

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The Kupffer cell (KC) inhibitor, gadolinium chloride ($GdCl_3$), protects the liver from a number of toxicants that require biotransformation to elicit toxicity (1,2-dichlorobenzene and CCl_4), as well as compounds that do not (cadmium chloride ($CdCl_2$) and beryllium sulfate). The mechanism of this protection is thought to result from reduced secretion of inflammatory and cytotoxic products from KC. However, since mediators released from KC can also alter cytochrome P450 activity, these experiments were designed to determine if $GdCl_3$ pretreatment alters P450 (levels and activity). In addition, how $GdCl_3$ pretreatment affects the response of isolated hepatocytes in primary culture to certain toxicants was determined. Male, Sprague-Dawley rats were given $GdCl_3$ (i.v., 10 mg/kg), and 24h later, livers were either processed for preparation of microsomes or for primary cultures of hepatocytes. $GdCl_3$ reduced total microsomal P450 as well as aniline hydroxylase activity by ~ 50% This alteration in P450 may explain why $GdCl_3$ pretreatment affected, differentially, the response of hepatocytes to $CdCl_2$ and CCl_4. While $GdCl_3$ decreased the toxicity of CCl_4 in hepatocytes (LC_{50} of 1.9 (control) vs. 3.8 ($GdCl_3$) ul), it had no effect on $CdCl_2$ toxicity (LC_{50} of 0.88 (control) vs. 0.82 ($GdCl_3$) uM). These results suggest that $GdCl_3$ produces multiple effects that may alter the susceptibility of hepatocytes to chemical induced toxicity. They further suggest that these protective effects of $GdCl_3$ may be chemical-dependent. (Supported by Center for Toxicology/Flinn Research Fellowship, NIEHS ES 06095 and Center Grant # P30 ES06694).

418 ROLE OF METALLOTHIONEIN (MT) EXPRESSION IN THE DEVELOPMENT OF TOLERANCE TO CADMIUM HEPATIC TOXICITY INDUCED BY ALL-*TRANS*-RETINOL *IN VIVO* AND IN ISOLATED HEPATOCYTES

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Cadmium chloride ($CdCl_2$) exposure can induce acute, lethal hepatocellular necrosis in rodents, as well as lesions in the testes, lung, kidney, and GI tract. Several substances can induce tolerance to the toxicity of Cd, including other metals. Recently, we have found that pretreatment with all-trans-retinol (vitamin A) greatly attenuates Cd toxicity in the rat, markedly reducing acute hepatotoxicity. However, the mechanism by which retinal induces tolerance to Cd is as yet undefined. In many cases the inducible Cd-binding protein, metallothionein (MT) is a essential aspect to the acquisition of Cd tolerance. Therefore, the objective of this study was to further characterize the effects of retinol on the hepatotoxicity caused by Cd and to elucidate possible mechanisms, including a possible role for MT in male Sprague-Dawley rats. Retinol (75 mg/kg/d, *p.o.*) or vehicle was administered for 7 d, and 24 hr later rats were given $CdCl_2$ (2.0 – 4.0 mg/kg, *i.v.*). MT was assessed by the Cd-Hemoglobin assay. Retinol pretreatment significantly attenuated the acute lung, liver, and testicular toxicity caused by Cd at 6, 24, and 48 hr postdosing. Furthermore, retinol pretreatment significantly increased MT in the liver by 7-fold. MT levels in other tissues, including the kidney and lung, were not effected by retinol. Although this increase in MT was much less than that induced by Cd, it was additive to the induction of Cd. In monolayers of primary isolated hepatocytes Cd induced toxicity was significantly reduced in hepatocytes isolated from retinol pretreated rats as compared to control. The dose response was shifted to the right and the LC_{50} was increased from 1.06 ± 0.06 to 2.38 ± 0.04 μM. Thus, induction of hepatic MT is an essential aspect of retinol-induced tolerance to Cd hepatotoxicity, and subse-

quent alteration of Cd distribution may reduce Cd toxicity in other tissues. (Supported in part by P30-ES-O6694, R01-ES-O6095, and a P&G Graduate Fellowship).

419 IMMUNOHISTOCHEMICAL LOCALIZATION OF HSP 25 AND HSP 70 INDUCTION IN MURINE LIVER AFTER ACUTE TREATMENT WITH CARBON TETRACHLORIDE OR BROMOBENZENE

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The effect of bromobenzene and carbon tetrachloride on heat shock protein (hsp) induction in the liver was examined using Western blotting and immunohistochemistry. Mice were administered a single dose of carbon tetrachloride (0.04 ml/kg, i.p.) or bromobenzene (0.4 ml/kg, i.p.) and the level of hsps in the liver analyzed 3, 6, 9, 12, 24, and 72 hours after the dose. As measured by Western blotting, neither hepatotoxicant altered the level of hsp60, hsc70, or hsp90 at any of the observation times. Carbon tetrachloride induced both hsp25 and hsp701 at 9, 12, and 24 hours. No induction was observed at 72 hours. Bromobenzene induced hsp25 and hsp701 weakly by 12 hours, and strong induction was seen at 24 and 72 hours. Immunohistochemical localization of hsp25 and hsp701 proved to be a more sensitive tool in evaluating hsp induction. Hsp25 and hsp701 induction after carbon tetrachloride treatment was exclusively located in cells with altered morphology at 3, 6, 9, 12, and 24 hours. By 72 hours, hsp induction was not evident. Hsp25 and hsp701 induction was observed 6, 9 and 12 hours after bromobenzene treatment, but was minimal compared to that seen at 24 hours. Induction at 24 hours was restricted to the periphery of the lesion and a layer of cells surrounding the lesion. Hsp induction at 72 hours was still apparent and was restricted to cells surrounding necrotic areas. The contrast between the pattern of hsp induction caused by carbon tetrachloride and bromobenzene suggests different relationships between toxicity and hsp induction for these two classical hepatotoxicants. [Supported by ES-07213]

420 EFFECT OF TACRINE ON ISOLATED HEPATIC MITOCHONDRIA

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Tacrine (1,2,3,4-tetrahydro-9-acridinamine monohydrochloride monohydrate), is a drug approved for the treatment of Alzheimer's disease and causes serum transaminase elevations indicative of hepatotoxicity in some patients. Hepatotoxicity has not been noted in animal safety studies and there is a need to understand the nature of functional liver changes. Preliminary work in *in vitro* models suggested that mitochondria may be involved in the etiology of the hepatotoxic effect. Therefore, the effect of tacrine on respiration in mitochondria isolated from rat, dog, monkey and human liver was examined. Tacrine produced significantly decreased respiratory control ratios (RCR) in all species at concentrations ranging from 5 to 25 μg/mL. Human mitochondria appeared to be more sensitive to tacrine. The hepatic mitochondrial changes were characterized primarily by increased State 4 respiration in rats and decreased State 3 respiration in humans. ADP/O (P/O) ratios were largely unaffected. Only human mitochondria showed a significant decrease at the highest concentration tested (50 μg/mL). Several metabolites of tacrine were examined as to their potential mitochondrial toxicity and all were found to be less toxic than the parent compound with the rank order of the monohydroxylated metabolites: tacrine > 4-OH = 7-OH >2-OH = 1-OH = velnacrine = no effect. *In vivo* administration of tacrine (20 mg/kg) for up to 20 days produced only minor mitochondrial effects with a paradoxical increase in RCR and P/O on Day 1 and decreased RCR on Days 9 and 20 consistent with the *in vitro* data. From these data we conclude that tacrine affects mitochondrial function which may contribute to the mechanistic basis for the observed hepatotoxicity.

421 ACTIVATION OF A NOVEL FORM OF PHOSPHOLIPASE A2 DURING LIVER REGENERATION

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Sponsor: R.D. Irons

Activation of phospholipase A2 (PLA2) activity occurs following cellular injury, such as that due to ischemia or carbon tetrachloride exposure, and in response to mitogenic stimulation by growth factors. Within 1 hour after partial hepatectomy, increased PLA2 activity specific for phosphatidylethanolamine (PE) was detected in cell-free homogenates prepared from the remaining liver tissue. Gel filtration chromatography revealed three peaks of PLA2 activity, one of which was identified as cPLA2. However, the majority of PE-specific PLA2 activity was due to a high molecular weight enzyme which was stimulated 5-fold during regeneration. This enzyme was selective for substrates containing arachidonic acid at the sn-2 position, and was inhibited by arachidonic acid analogues previously shown to be reasonably selective for cPLA2. However, although it showed a preference for calcium over other divalent cations, it required higher concentrations of calcium for maximal activation than cPLA2, and eluted differently from a MonoQ anion exchange column. A third, low molecular weight peak of PLA2 activity was also stimulated, but was not characterized further. The activation of the novel, high molecular weight form of PLA2 may represent an early event in the proliferative response of hepatocytes.

422 ENHANCED TRANSCRIPTION OF HEPATIC ADHESION MOLECULE GENES DURING ENDOTOXEMIA

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Adhesion molecules are critical for leukocyte migration to sites of inflammation. The change of hepatic mRNA levels for intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1) and P-selectin were determined by Northern blot analyses after administration of 100 μg/kg *Salm. abortus equi* endotoxin (ET) in mice. In the ET-sensitive strain C3Heb/FeJ, which generated cytokines (TNF-α, IL-1) in response to ET, hepatic mRNA levels of all CAMs increased after ET. For ICAM-1 and VCAM-1 mRNA, the highest levels were observed at 90 min with a gradual decline during the next 6 h. For P-selectin, the mRNA levels increased up to 4 h after ET and were no longer detectable at 7 h. In contrast, the ET-resistant strain C3H/HeJ, which did not produce any cytokines, did not show any increase of CAM mRNAs in response to ET at all time points. Injection of murine recomb. TNF-α (20 μg/kg), IL-1α (13 μg/kg) or IL-1β (23 μg/kg) induced CAM mRNA in the liver to a similar degree in both strains of mice. We conclude that there is a rapid response to upregulate ICAM-1, VCAM-1 and P-selectin in the liver after endotoxin injection; the enhanced transcription is induced by TNF-α and IL-1.

423 ENDOTOXIN-INDUCED ACTIVATION OF NUCLEAR FACTOR-κB (NF-κB) AND HEPATIC E-SELECTIN GENE TRANSCRIPTION

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NF-κB is a ubiquitous transcription factor implicated in the activation of rapid-response genes during inflammation. To investigate the role of NF-κB in E-selectin gene transcription in the liver *in vivo*, Fischer rats were injected with 0.5 mg/kg *Salm. enteritidis* endotoxin (ET) and liver cells were isolated 1 and 5 h later. Using the electrophoretic mobility shift assay, no NF-κB activation was found in intact control liver; a minor activation was detected in hepatocytes and endothelial cells, however, Kupffer cells showed a substantial activation after the isolation procedure. No E-selectin mRNA was found in any control cell type. One hour after ET, massive NF-κB activation was seen at the whole liver level and in each individual cell type. E-selectin mRNA was found only in endothelial cells and Kupffer cells. At 5 h, NF-κB activation was strongly attenuated and E-selection mRNA was no longer detectable in these cells. We conclude that NF-κB activation is a necessary step to induce E-selectin gene transcription in liver endothelial cells and Kupffer cells. The lack of mRNA synthesis in hepatocytes suggests that factors other than NF-κB regulate cell type specific E-selectin gene expression.

424 AGE-RELATED DIFFERENCES IN TGF-α AND PROTO-ONCOGENES EXPRESSION IN RAT LIVER AFTER CD + CCl_4 TREATMENT

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Recent studies from our laboratory have demonstrated that mRNA expressions for transforming growth factor-α (TGF-α) and proto-oncogenes increased in age- and time-dependent manner after a single low dose of CCl_4. The objective

of the current study was to investigate if a combination of chlordecone (CD) and CCl_4 treatment modulates gene expression patterns seen in CCl_4 treated rats. Postnatally developing (20-d) and adult (60-d) male S-D rats were challenged with a single low dose of CCl_4 (100 μl/kg, ip) or corn oil after pretreatment with 10 ppm dietary CD for 15 days. Liver samples were collected during a time-course (0–48 hr) after CCl_4 to examine mRNA expressions for TGF-α and proto-oncogenes (*c-fos H-ras*, and *K-ras*). There was no significant increase in TGF-α, gene expression regardless of age. In contrast, appreciable gene expressions of *c-fos, H-ras*, and *K-ras* were evident after CCl_4 in both age groups, with earlier response in 20-d rats and later time points in 60-d rats as detected by slot-blot analysis. Results of the study revealed about 2- and 3-fold increases in *c-fos* gene expression in 20- and 60-d rats, respectively, 1 hr after the administration of CCl_4 compared to their untreated controls. There were about 1.5-fold increases in *H-ras* and *K-ras* during maximal induction in both age groups. The overall findings of this study indicate that TGF-α did not seem to have a role in the resiliency of 20-d rats to CD + CCl_4. On the other hand, earlier response in the expressions of proto-oncogenes (*c-fos, H-ras*, and *K-ras*) appeared to play a role in the survival of 20-d rats treated with CD + CCl_4 combination that resulted in 100% mortality in 60-d rats. (Supported by NIA)

425 2D-ELECTROPHORETIC ANALYSIS OF THIOACETAMIDE-INDUCED PROTEIN CHARGE MODIFICATION IN RAT LIVER

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Thioacetamide (TA) has been reported to form an intermediate that binds to proteins forming acetylimidolysine derivatives responsible for TA's hepatotoxic effects. TA has also been reported to cause cell death via both apoptosis and necrosis. The objective of this study was to investigate the effect of TA exposure on protein charge modifications in the rat liver and second to study the role of these molecular correlates in the regulation of cell death. Male Sprague-Dawley rats (200–225g) were divided into 4 groups and treated intraperitoneally with a 12-fold dose-range of TA (50, 150, 300 and 600 mg TA/kg) dissolved in water. Using whole liver extracts, alterations in the hepatic protein pattern following treatment was studied using two-dimensional polyacrylamide gel electrophoresis (2DE) and computerized image analysis. The results indicate that protein charge modification was clearly evident as early as 2 hr with the lowest dose of 50 mg TA/kg. At this dose and time, ER proteins calreticulin, grp78, and ER60 exhibited acidic charge variants, effects made more prominent with dose and time. Elevation of charge modification indices (CMI) by TA reached a peak between 4 and 6 hr. While CMI either leveled off or declined in the lower two doses of 50 and 150 mg TA/kg, it continued to remain elevated with the higher doses of 300 and 600 mg TA/kg. This dichotomy in CMI elevation corresponds to cell death patterns observed with a similar dose-range of TA, where lower doses (50 and 150 mg TA/kg) predominantly cause cell death via apoptosis and higher doses cause cell death via necrosis. Delayed charge modification observed in cytosolic hsc70 with the 300 and 600 mg TA/kg treatments suggests that the reactive metabolite(s) slowly leak in from the ER. The absence of alteration in the mitochondrial proteins hsp60 and grp75 suggests that TA has no effect on the mitochondrion and that its effects are primarily confined to the endoplasmic reticulum. The observed changes may be indicators of bioactivation and adduct formation and also may be signaling events in the regulation of the mode of cell death. Supported by AFOSR Grant #F49620-93-1-0297.

426 EFFECT OF A KETONE ON MANGANESE, BILIRUBIN AND LIPID CONTENT OF HEPATIC MEMBRANES

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In manganese (Mn)-bilirubin (BR) induced cholestasis, Mn and BR can be taken up by bile canalicular (BCM) and sinusoidal plasma membranes (PM). Since ketones can enhance the cholestatic properties of Mn-BR, the effects of methyl isobutyl ketone (MiBK) on BCM and PM Mn and BR content was investigated. Male Sprague-Dawley rats were pretreated *in vivo* with MiBK p.o. (7.5 mmol/kg) for 3 days before the cholestatic regimen (^{54}Mn 4.5 mg/kg i.v. followed 15 min later by BR 25 mg/kg i.v.). One hr after BR, liver cell plasma membranes were isolated; ^{54}Mn and BR contents were measured. Cholesterol and phospholipid content of BCM and PM fractions was investigated and fluidity assessed with fluorescent probes (DPH and TMA-DPH). MiBK had no significant effect on Mn or BR incorporation in BCM or PM. BCM cholesterol content, however, was significantly increased compared to controls (3-fold). Phospholipids were unaffected in both fractions. The fluidity results indicate that MiBK pretreatment had no important effect on BCM or PM. Therefore, altered Mn or BR incorporation in BCM does not seem to be a mechanism involved in MiBK-potentiated Mn-BR cholestasis, whereas altered lipid content correlates better with cholestasis. (Supported by the MRC, the Canadian Network of Toxicology Centres and the Canadian Liver Foundation)

427 PHENOTYPIC CHARACTERIZATION OF LONG-TERM CULTURES OF PRIMARY HUMAN HEPATOCYTES: EFFECTS OF CULTURE CONDITIONS AND DURATION

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A variety of markers of hepatic parenchymal cell differentiated function were assessed using primary isolates of human hepatocytes cultured under a variety of conditions for up to 4 weeks. Hepatocytes were obtained from the Anatomic Gifts Foundation and were isolated by conventional collagenase perfusion techniques. Cells were plated at varying densities on either 6-well or 12-well collagen-coated plates. In some cases, the cells were overlaid with extracellular matrix (Matrigel). The secretion of albumin and apolipoproteins Al and B was monitored by ELISA. The activities of cytochrome P450 3A4 (CYP3A4) in the basal state or after phenobarbital or rifampicin pretreatment were monitored by assessing testosterone 6B-hydroxylation. The ability of the hepatocytes under various conditions to carry out glucuronidation and sulfation reactions was assessed by monitoring the conjugation of hydroxycoumarin. The levels of CYP3A4 and cytochrome P450 reductase were also monitored by Western blot. The results show considerable variability among preparatins in all parameters, but clear trends in the data were evident: Maximal recovery of differentiated function as assessed by protein secretion, P450 levels and P450 inducibility was not seen in most hepatocyte preparations until after 7-10 days in culture and persisted for at least an additional 14 days. At lower cell densities, extracellular matrix enhanced the differentiated function of the hepatocytes. At higher cell densities the beneficial effects of extracellular matrix were diminished. Finally, in the presence of dexamethasone, phenobarbital and rifampicin pretreatment lead to 2-10 fold increases in CYP3A4 activity.

428 DAUNORUBICIN (DAU) AND CYCLOSPORINE A (CsA) PROTECT AGAINST ESTRADIOL-17β-GLUCURONIDE ($E_2$17G) CHOLESTASIS

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$E_2$17G inhibits bile flow following its intravenous administration. It's transport in canalicular membrane vesicles is ATP-dependent and inhibited by substrates and modulators of P-glycoprotein (P-gp) (Cancer Res 53:5382) such as DAU and CsA. The present studies were designed to determine if DAU and CsA alter $E_2$17G cholestasis in the single pass perfused rat liver. Methods. $E_2$17G (1–16 μmol; bolus dose) was infused via the portal vein in the presence or absence of DAU (1.5 μM) or CsA (6 μM) in the perfusate and bile flow (μl/min/g liver) measured. *, $p < .05$; $n = 3-5$. Results. $E_2$17G (2 μmol) decreased bile flow from 1.44 ± 0.07 to 0.39 ± 0.06 (*) at 10 min and to 0.98 ± 0.15 (*) at 60 min. In the presence of DAU, bile flow decreased from 1.48 ± 0.15 to 1.31 ± 0.31 (10 min) and 1.29 ± 0.22 (60 min). In the presence of CsA, $E_2$17G (16 μmol) decreased bile flow from 1.49 ± 0.05 to 1.21 ± 0.06 (10 min) and 1.06 ± 0.23 (60 min). DAU shifted the log-dose response curve for $E_2$17G cholestasis to the right 4-fold in an almost parallel manner, while CsA shifted the curve to the right in a markedly non-parallel manner. Conclusions. DAU and CsA protect against $E_2$17G cholestasis, supporting the hypothesis that $E_2$17G is a substrate of P-gp and that P-gp may be a target of $E_2$17G-induced cholestasis.

429 PHOSPHOLIPID ACCUMULATION IN GALLBLADDER EPITHELIUM: A UNIQUE RESPONSE TO A CATIONIC AMPHIPHILIC DRUG

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During safety evaluation of a pyrrolopyrimidine drug candidate, U-101033E, inflammatory and necrotizing lesions were found in the gallbladder and large, intrahepatic bile ducts of dogs given the compound orally. Using electron microscopy, the gallbladder epithelium of drug-treated dogs was found to contain numerous lamellar bodies. These bodies consist primarily of phospholipids and form as a result of altered membrane turnover. They are hallmark structures of phospholipidosis and are induced by a variety of structurally and pharmacologically dissimilar compounds collectively referred to as cationic amphiphilic drugs (CADs). Although the U-101033E fits the structural criteria for a CAD, biliary tract changes were not previously reported for other CADs. Similar findings occurred in the gallbladders of monkeys and guinea pigs treated orally with U-101033E, but not in mice, gerbils, hamsters, or rabbits. The susceptibility to drug-induced lesions in the gallbladder coincided with specific ultrastructural features of the epithelium. Susceptible species had an abundance of lysosomes juxtaposed to extensive Golgi structures indicating these cells to be quite active in secretion and degradation. The epithelium of resistant species had a paucity of lysosomes. Since the gallbladder epithelium of humans is ultrastructurally similar to that of the susceptible species, our results suggested that humans would develop similar gallbladder changes if exposed to this compound.

430 EXAMINATION OF METABOLIZING CAPABILITY IN RAT STRAIN DIFFERENCES IN CARBON TETRACHLORIDE HEPATOTOXICITY

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Fischer-344 (F-344) and Sprague-Dawley (SD) rats differ in their response to hepatotoxicants. Relative to SD rats, carbon tetrachloride (CCl4) hepatotoxicity is greater in both age-matched (44, 64 and 120 day old) and weight-matched (~200 g) F-344 rats, based on elevation of serum enzymes reflective of hepatic injury and hepatic histopathology. As hepatic CYP2E1 is thought to be the principal P450 isozyme responsible for CCl4 metabolism, the objective of the present study was to investigate CYP2E1 activity in these two rats strains. Hepatic CYP2E1 activity, expressed as p-nitrophenol hydroxylase activity (pNC)/mg microsomal protein/min, was not significantly different between the two strains in 44- and 120-day-old age-matched rats and in the weight-matched rats. This confirms our previous finding with 64-day-old age-matched rats. Comparisons other than those based on specific activity may be appropriate where differences in either hepatic microsomal protein concentration or liver size might be expected. Expressed as pNC/gm liver/min, hepatic CYP2E1 activity was significantly greater in F-344 rats relative to SD rats in all three age-matched groups (44, 64 and 120 days) but not in the weight-matched rats. Due to significantly greater liver weight (gm) in the SD rats, hepatic CYP2E1 activity expressed as pNC/liver/min was significantly greater in the SD rats relative to their age-matched F-344 counterparts. In summary, hepatic CYP2E1 activity expressed per mg microsomal protein or per liver cannot explain the observed strain differences in CCl4 toxicity. Although CYP2E1 activity per gm liver is consistent with strain differences in the age-matched rats, it is inconsistent with strain differences in the weight-matched rats. This highlights the importance of using both age- and weight-matched animals in strain comparisons. In conclusion, these data suggest differences in CYP2E1-mediated metabolism may not underlie variation in CCl4 toxicity due to rat strain. (This abstract may not reflect EPA policy.)

431 EXAMINATION OF THE EFFECT OF GAVAGE VEHICLE ON CARBON TETRACHLORIDE HEPATOTOXICITY IN F-344 RATS

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Both gavage vehicle and gavage volume have been shown to modulate haloalkane hepatotoxicity. In adult male F-344 rats, oil vehicle administration increases the acute hepatotoxicity of both chloroform and bromodichloromethane relative to aqueous vehicle administration. The effect of vehicle of administration on carbon tetrachloride (CC14) hepatotoxicity is not clear-cut. In male Sprague-Dawley (SD) rats, oil administration has been reported to decrease CC14 acute hepatotoxicity (Kim et al., 1990). Raymond and Plaa (1994) found no significant differences in slopes for dose-response curves for CC14 hepatotoxicity between oil and aqueous vehicles; however, a trend of enhanced toxicity with corn oil was seen at low toxicity incidence. Vehicle of administration has been reported not to affect the subchronic hepatic toxicity of CC14 in male SD rats (Koporec et al., 1995). F-344 and SD rats differ in their sensitivity to CC14; in both age-matched and weight-matched animals, CC14 hepatotoxicity is more severe in F-344 rats. Therefore, the objective of the present study was to examine gavage vehicle effects in F-344 rats. Adult, male F-344 rats (~70 days old) were gavaged with 0, 0.02, 0.10, 0.50 and 2.50 ml CC14/kg in corn oil or 10% Alkamuls EL-620® solution at a gavage volume of 5 ml/kg. Hepatotoxicity was assessed 24 hr later. CC14 produced dosage-dependent hepatotoxicity in both vehicles. Based on serum activities of alanine and aspartate aminotransferase and sorbitol dehydrogenase, vehicle of administration had little or no effect on CC14 hepatotoxicity. Except at 0.5 ml CC14/kg, where more severe hepatocellular degeneration was seen in rats receiving CC14 in oil, differences in hepatic histopathology due to vehicle were not readily apparent. In conclusion, gavage vehicle has little apparent effect on CC14 hepatotoxicity in male F-344 rats. (This abstract may not reflect EPA policy.)

432 THE PROTECTION AGAINST CARBON TETRACHLORIDE HEPATOTOXICITY BY OLEANOLIC ACID IS NOT MEDIATED BY METALLOTHIONEIN

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Oleanolic acid is a triterpenoid compound that has been shown to protect against liver injury produced by some hepatotoxicants. This study was designed to examine the hepatoprotective effects of oleanolic acid against carbon tetrachloride-induced liver injury, and the role of metallothionein (MT) in the protection. Oleanolic acid pretreatment (100–400 μmol/kg, sc) protected CF-1 mice and Sprague-Dawley rats from carbon tetrachloride-induced liver injury in a dose- and time-dependent manner, as evidenced by serum alanine amino-transferase and sorbitol dehydrogenase activities, as well as by histopathology. The protection was not evident until animals were pretreated with oleanolic acid 14 hr prior to carbon tetrachloride administration. MT-I and II knock-out (MT-null) and corresponding control (129 × C57) mice were used to examine the role of MT in oleanolic acid-induced protection. Oleanolic acid (200 μmol/kg, sc) treatment increased hepatic MT 20-fold in control but not in MT-null mice, but it equally protected against the hepatotoxicity of carbon tetrachloride in both control and MT-null mice. These data indicate that the protection against carbon tetrachloride hepatotoxicity by oleanolic acid is not mediated through induction of MT. (Supported by NIH Grant ES-06190)

433 EFFECT OF IRON-OVERLOAD ON POLYAMINE OXIDASE ACTIVITY IN RAT LIVER

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Iron overload is associated with cell injury, fibrosis and cirrhosis in liver. The aim of this study was to investigate the effect of iron overload on polyamine oxidase activity (PAO) and ornithine decarboxylase (ODC) activities, in hearts and livers of rats. Both ODC and PAO are implicated in cellular growth, differentiation and cell death. But their role in iron overload mediated tissue injury is not known. ODC is an initial rate-limiting enzyme in the synthesis of polyamines (putrescine, spermidine and spermine). PAO oxidatively deaminates polyamines resulting in the formation of precursors of polyamines, 3-acetamido-propanal and H_2O_2. In these experiments, the rats were treated with iron-dextrans (100 mg/kg/day, i.p.) and sacrificed at day 7. Livers and hearts were removed for the determination of (i) PAO activity by the release of H_2O_2 from N-^{1}-acetyl-spermine as a substrate and conversion of homovanillic acid into a fluorescently detectable compound, (ii) ODC activity as a release of $^{14}CO_2$ from ^{14}C-ornithine and (iii) lipid peroxidation by the thiobarbiturate reaction. Iron overload led to 5.6 fold increase in PAO activity ($p < 0.001$) and 3 fold increase in lipid peroxidation in liver ($p < 0.0001$). The basal ODC activity of liver was not significantly changed by iron overload. In hearts, iron overload caused no significant changes in PAO or ODC activity and lipid peroxidation. These results suggest a potential role for PAO in the iron-overload mediated cell injury in rat liver.

434 A STOCHASTIC MODEL OF CELL TOXICITY: COMPARISON WITH CELL CULTURE RESULTS

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In an effort to develop a mathematical description of organ toxicity, the salient cell biology of individual cells in tissue has been abstracted into a multistate model of interaction between individual cells and their environment. In order to parameterize and test the model using cell culture data, the original model has been reduced to a minimal three state stochastic model. This model describes cell killing and modifications in cell cycle dynamics in response to toxic chemical exposure. The three model states, defined by specific biochemical markers, are dividing, new, and mature. To begin model refinement, type WB liver hepatocytes in culture have been exposed to varying doses of acetaminophen under maximal growth conditions. Time course data for the number of cells in each state have been measured. The dynamics of cell death and regrowth under these conditions have been analyzed by fitting the model to the data. Results of cell culture experiments, derived model parameters, and quality of fit will be presented. Implications of these experiments for toxicity modeling will be discussed.

435 CAFFEINE-INDUCED POTENTIATION OF ALLYL ALCOHOL HEPATOTOXICITY IN RATS. A MORPHOLOGICAL STUDY

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Our previous studies have shown the potentiating effects of caffeine (CF) on allyl alcohol(AA)-induced hepatotoxicity in adult male Sprague-Dawley rats both in vitro (Toxicologist 14:370, 1994) and *in vivo* (Toxicologist *15*:219, 1995) from biochemical studies. The objective of this study was to verify, using morphological approaches, our previous results. Adult male Sprague-Dawley rats were treated i.p. with AA (1.8 mg/kg) and the animals were sacrificed 24 h later. There was minimal hepatic damage consisting of occasional necrotic hepatocytes and inflammatory infiltrate surrounding the portal tract area. Combined *in vivo* treatment of rats with AA (1.8 mg/kg, i.p.) and CF (150 mg/kg, oral) showed greater severity of such hepatic lesions compared to AA alone-treated rats. This consisted of necrosis of periportal hepatocytes and severe accumulation of inflammatory infiltrate in necrotic zones. The combined treatment with AA and 75 mg/kg of CF however did not produce any such change compared to AA alone. Pretreatment of rats with 4-methylpyrazole (an inhibitor of alcohol dehydrogenase) (0.5 mmol/kg, i.p.) for 30 min markedly decreased the severe liver damage due to AA plus CF (150 mg/kg) treatment. Pretreatment of rats with phenobarbital (PB) (80 mg/kg, 3 days) showed minimal damage to moderate necrosis and inflammatory response after treatment with AA and CF (75 mg/kg). But the severe liver damage due to AA plus CF (150 mg/kg) treatment was found to be further enhanced to a maximum after PB pretreatment. These data thus confirmed our previous findings based on biochemical studies that CF potentiation of AA hepatotoxicity depends on (1) conversion of AA to acrolein and (2) metabolic activation of CF by PB-inducible cytochromes P450. (Supported by CNTC and World University Services of Canada)

436 THE ROLE OF KUPFFER CELLS AND CYTOCHROME P450 IN ONE DAY RETINOL POTENTIATION OF CCL_4-INDUCED LIVER INJURY

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Retinol pretreatment for 7d potentiates CCl_4-induced liver injury in male, SD rats by a mechanism that involves activation of Kupffer cells (KC). These activated KC secrete products (superoxide anion, hydrogen peroxide, etc.) that promote progression of injury. Recently, it was observed that a single dose of retinol can potentiate CC14 liver injury. To determine the role of KC in the 1d model, the KC inhibitor, $GdCl_3$ (i.v. 10 mg/kg), was administered to rats just prior to pretreatment with retinol (75 mg/kg, *po*). CCl_4 (0.2 ml/kg, *ip*) was administered 24th later. At 24h after CCl_4, plasma ALT activities were 220 ± 51, 2687 ±366 and 1133 ± 213 U/L in the CCl_4, retinol + CCl_4, and retinol + GdC13 + CCl4 groups, respectively). Quantitative morphometric analysis of H&E stained liver sections confirmed these alterations in the degree of liver injury. Because $GdCl_3$ did not completely attenuate retinol potentiated injury (i.e. only 60%), the effect of retinol on CYP 2El activity (the major form that metabolizes CC14) was determined. At 24h, retinol pretreatment elicited a 2.4-fold increase in aniline hydroxylase activity which was confirmed by densitometric analysis of western blots for CYP 2E1. Interestingly, neither aniline hydroxylase activity nor 2E1 levels were elevated 48h following this single dose of retinol. Correspondingly, when rats were given retinol 48h prior to CCl_4, ALT activity was 159 ± 66 U/L (no potentiation). The results of these studies suggest that multiple mechanisms may be involved in the 1d retinol potentiation of CCl_4. (Supported by Center for Toxicology/Flinn Research Fellowship, NIEHS ES 06095 and Center Grant # P30-ES06694).

437 THE USE AND MISUSE OF TOXICOLOGY EVIDENCE IN THE COURTS

G S Edwards and F Welsch, *ToxiCon Associates, Natick, MA; CIIT, Research Triangle Park, NC*

In recent years several instances of perceived misuse of scientific evidence have engendered calls for reform concerning qualifications of expert witnesses and admissibility of their testimony. The 1993 Supreme Court *Daubert* decision (a case alleging that the anti-nausea drug Bendectin[R] caused birth defects) changed the previous standards for consideration of expert depositions. The Federal Judicial Center recently published The Reference Manual for Scientific Evidence to augment that decision and to help judges facing complex scientific issues. This book provides guidelines to aid judges in deciding on the qualifications of expert witnesses and the admissibility of their testimony. The first speaker is the author of the Manual's chapter on toxicology. Others on the program will discuss examples of the use and misuse of toxicology evidence, ethical considerations for expert witnesses, the evolving standards for scientific evidence, and a practical primer for toxicologists serving as expert witnesses. The purpose of this workshop is to familiarize SOT members with the emerging legal standards for scientific evidence, particularly as they apply to toxicology. Furthermore, the objective is to provide SOT members with some advice about what to expect should they be called upon to provide an expert opinion.

438 TOXICOLOGICAL IMPLICATIONS OF CO-EXPOSURE TO ULTRAVIOLET LIGHT AND PHARMACEUTICALS

Chairpersons: A L Kraus and R E Osterberg. *The Procter & Gamble Company, Cincinnati, OH, and the U.S. Food and Drug Administration, Rockville, MD*

Historically, phototoxicity surfaced with sulphonamides and tetracyclines in the 1940s–1960s. Recent evidence of photoactivity from a variety of drug classes and the separate but concerning increasing incidence of skin cancer has elevated concerns of combined drug-ultraviolet light interactions. As a result, there are increasing regulatory requests for phototoxicology data. Some of these requests are for data from models designed to predict possible human response, while others are from models with limited, if any, human health response correlates. Given the expansion of awareness to phototoxicities with compounds such as psoralins, phenothiazines, and, more recently, fluoroquinolones, significant discussion on the use of screening procedures and development tests is under way. While some models appear to provide helpful correlates to potential human response (i.e., photoirritation and photoallergy), others are less tested and more controversial (i.e., photocarcinogenicity). The U.S. Food & Drug Administration is currently evaluating these issues and would value scientific discussion and input into the value of various photobiological and phototoxicological approaches. This session will overview available approaches in photochemistry, photoirritation, photoallergy, photoimmunology, and photocarcinogenicity. The presentations and group discussions will seek to identify models of significant value and areas of uncertainty or concern that could be aided by additional research. While this workshop will provide an overview of this emerging area with primarily a U.S. FDA and drug product perspective, discussion may apply to other arenas.

439 OSTEOTOXICITY AND INTERVENTION PROTOCOLS: INTRODUCTION

E Jeffery, M H Bhattacharyya. *University of Illinois, Urbana, IL; Argonne National Laboratory, Argonne, IL*

The majority of the body burden of xenobiotic minerals such as lead, fluoride, and strontium is found associated with the skeleton. Bone has long been recognized as a storage site for xenobiotic minerals. The possibility that these

xenobiotics might be toxic to bone, or even be released during bone resorption to produce systemic toxicity, is a relatively recent consideration. When industrial chemicals or new drugs are screened for toxicity, no evaluation of osteotoxicity is required. Development of therapies for osteoporosis is currently a very active area in the pharmaceutical field. In this area, chemicals are specifically chosen for their effects on bone physiology, and methods have evolved to evaluate osteotoxicity. Our first symposium speaker is a highly respected member of the bone research community who will provide detailed insight into the role of vitamin D in normal bone growth and remodeling. This topic will feed directly into the second presentation, which addresses the osteotoxicity of lead, focussing on the cellular and molecular biology of lead interactions with the vitamin D endocrine system. Cadmium osteotoxicity will be addressed next, with unique insights provided at all levels, from humans to whole animals to organ and cell culture systems, including a view toward identifying susceptible populations. The final presentation addresses the issue of therapeutic protocols for treating osteoporosis, providing insight into current treatment options and toxicological and pharmacological aspects of developing new protocols that meet FDA guidelines.

440 ROLE OF VITAMIN D IN BONE

H F DeLuca. *Department of Biochemistry, University of Wisconsin, Madison, WI, USA.* Sponsor: M H Bhattacharyya

Vitamin D, through its functional hormone, 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), has a very profound role in bone function and metabolism. First, it is required to elevate plasma calcium and phosphorus to levels required to support mineralization of bone. Secondly, osteoblasts are a major target site where, through its receptor, 1,25-$(OH)_2D_3$ directly enhances transcription of genes that encode the bone proteins, osteocalcin and osteopontin. In addition, vitamin D is required for the mobilization of calcium from bone to plasma. This process requires the action of the parathyroid hormone. The parathyroid hormone also binds to the osteoblasts and neither 1,25-$(OH)_2D_3$ nor parathyroid hormone bind to the osteoclast. Undoubtedly a signal is emitted from the osteoblasts in response to these hormones to existing osteoclasts to activate bone resorption. Furthermore, vitamin D plays an essential role in the differentiation of promyelocytes to monocytes and further to the formation of osteoclasts. Thus, 1,25-$(OH)_2D_3$ and parathyroid hormone initiate skeletal modeling and remodeling. Finally, 1,25-$(OH)_2D_3$ acts directly through its receptor on the preproparathyroid gene to suppress expression of parathyroid hormone. Thus, 1,25-$(OH)_2D_3$ must be regarded as a major hormone regulating bone metabolism and function. The molecular events involved in 1,25-$(OH)_2D_3$-induced gene expression will also be presented.

441 BONE AS A TARGET ORGAN FOR LEAD TOXICITY

J G Pounds. *Institute of Chemical Technology, Wayne State University, Detroit, MI, USA*

The skeleton is a central feature of lead toxicity as (a) the principal reservoir and dosimeter of lead, (b) an internal source of lead exposure, and (c) a target for lead toxicity. The systemic and cellular metabolism of Ca and Pb and the regulation of function of calciotropic hormones are complex and tightly linked. Perturbation of the vitamin D (VD_3) endocrine system has been used as a biomarker for lead toxicity. Experiments conducted in an osteoblast cell line (ROS 17/2.8) characterize the effects of Pb on VD_3-dependent gene function. RIA and Western blot analyses demonstrate that low concentrations of Pb, without effect on synthesis of total protein, RNA, or collagen, impair the synthesis of VD_3-induced osteocalcin, an acidic, Ca-binding, bone matrix protein. Osteocalcin mRNA decreases along with osteocalcin protein. Sensitivity to Pb segregates with the VD_3 response element of the osteocalcin promoter in transient transfections of chimeric rat and human osteocalcin promoter/CAT reporter genes. Impaired VD_3-induced osteocalcin synthesis in Pb-treated cells is not due to a decrease in vitamin D receptors as determined by a hydroxyapatite binding assay. Stimulation of ROS cells with VD_3 induces $[Ca^{2+}]_i$ transients and oscillations that are dampened by preexposure to 10 μM lead. Pb also activates PKCβ, which may couple the genomic and non-genomic activities of VD_3, since both transcriptional activation of the VD_3 receptor and $[Ca^{2+}]_i$ oscillations are modulated by PKC. Thus the Pb effects on osteocalcin are at the transcriptional level and mediated through the VD_3 receptor by perturbation of signal-transduction processes regulating receptor activity. These results will be related to lead effects on skeleton growth and development in humans.

442 CADMIUM OSTEOTOXICITY: INCREASED SUSCEPTIBILITY IN FEMALES

M H Bhattacharyya. *Center for Mechanistic Biology and Biotechnology, Argonne National Laboratory, Argonne, IL, USA*

Cadmium was first recognized as an osteotoxin in the 1940s, when cadmium exposure was identified as one cause of a severe, demineralizing bone disease—Itai-Itai disease—among residents of Toyama Prefecture in Japan, and of bone fractures among cadmium workers in France. However, the lack of a convincing animal model for cadmium-induced bone loss has fueled debate on whether cadmium is indeed an important osteotoxic agent. Recently, research has shown that susceptibility to cadmium-induced bone loss is significantly impacted by the physiological state of female animals, explaining in part why 95% of Itai-Itai cases were postmenopausal women with a history of multiple childbirths (avg., 6 children). Data will be presented that (1) clarify the role of cadmium as an important osteotoxin, (2) identify females as "susceptible individuals", (3) distinguish between renal vs. skeletal effects of cadmium and (4) evaluate molecular mechanisms for osteotoxic responses to cadmium, including those involving cyclooxygenase induction and prostaglandin synthesis in osteoblasts, the cells that form new bone and signal osteoclasts to erode old bone. Work supported by NIH Grant No. ES-04816 and by the U.S. Department of Energy, Office of Health and Environmental Research, under Contract No. W-31-109-ENG-38.

443 CURRENT TRENDS IN TREATMENT AND PREVENTION PROTOCOLS FOR OSTEOPOROSIS

N A Sacco-Gibson. *Clinical Bone and Mineral Research, Procter and Gamble Pharmaceuticals, Cincinnati, OH, USA.* Sponsor: E Jeffery

Osteoporosis is a decrease in which bone mass (bone material density) decreases, leading to impaired skeletal strength and increased fracture incidence. Although most prevalent among postmenopausal women, osteoporosis occurs in elderly men and in patients being treated with high-dose corticosteroids. Risk factors include not only estrogen deficiency, but also lifestyle, nutrition, genetic and environmental factors. By the early part of the 21st century, there will be greater than 100MM women at risk for osteoporosis, with as many as 20% having osteoporosis-related fractures. Useful therapies either decrease the abnormally increased rate of bone loss, or increase the amount of bone being formed in order to maintain a bone mass above fracture threshold. Evaluation of new therapies requires characterization of biochemical markers of bone turnover, assessment of fracture prevalence and incidence, and determination of bone mineral density changes in both preclinical and clinical studies, in addition to toxicological evaluation of potential side effects in hematologic, renal, and hepatic systems. This presentation will provide insight into current options for treatment of osteoporosis and the pharmacological and toxicological evaluations involved in development of new therapeutic measures, from the preclinical research stage to FDA approval of a new product or protocol.

444 DIETARY AND ENVIRONMENTAL ESTROGENS AND ANTIESTROGENS: COMPLEX ISSUES

W. G. Helferich, S. Safe, J. Yager. *Michigan State Univer., E. Lansing, MI, Texas A&M, College Station TX, Johns Hopkins Univer., Balt., MD*

Many dietary chemicals have been classified as estrogens and antiestrogens. The estrogenic potential of plant chemicals (phytoestrogens) was first described approximately fifty years ago when it was observed that sheep grazing on clover showed clinical signs of hyperestrogenism. The symptoms included: milk excretion from virgin females, milk excretion from castrated males and infertility. Although phytoestrogens are weak estrogen agonists they are consumed by both animals and humans at levels high enough to elicit estrogenic responses in experimental animal models. Additionally, it has been speculated that these weak agonists may act as antagonists to endogenous estradiol and act beneficially with regard to certain chronic diseases such as estrogen-dependent breast cancer. Asian populations which consume high levels of phytoestrogens in foods, such as soy and its derived products, have a relatively low incidence of breast cancer and other hormone-dependent cancers. Since the majority of human breast cancers are dependent

on estrogen, it appears the consumption of an estrogen agonist would present some risk rather than be preventative in women consuming phytoestrogens. This paradox of consumption of foods containing phytoestrogens and decreased levels of breast cancer will be discussed by the first two speakers. Recently chemicals which act as aromatic hydrocarbon receptor (AhR) agonists have been shown to be antiestrogenic in both in vivo and in vitro models. Potential mechanisms for the antiestrogenicity of these compounds will be discussed by the third speaker. Various environmental agents can alter the metabolism of both xenoestrogens and endogenous estrogens. The fourth speaker will discuss the role of increased metabolism to catechols and the resulting increased oxidative damage in estrogen-induced carcinogenesis.

445 FOOD PHYTOESTROGENS; AN ABUNDANCE OF WEAK ESTROGEN AGONISTS

W. G. Helferich. *Department of Food Science & Human Nutrition, Michigan State University. East Lansing, MI*

Human foods and animal feeds contain an abundance of phytoestrogens which are chemicals with low affinity for the estrogen receptor and are capable of inducing several estrogenic responses in animals and humans. Estrogen agonists may be detrimental to women at high risk for breast cancer. However, these estrogen-like chemicals may be beneficial to post-menopausal women with regard to bone density loss and risk of cardiovascular disease. Our research has focused on genistein, an abundant phytoestrogen in soy products. We have conducted *in vitro* studies using estrogen-responsive human, breast cancer (MCF-7) cells and demonstrated that genistein will induce expression of estrogen-responsive genes at concentrations as low as 100nM. Blood concentrations of genistein ranging from 1–3μM have been observed in humans consuming soy containing diets, suggesting that the bioavailability of genistein from soy products is sufficient to elicit an estrogenic response in humans. We have also conducted *in vivo* feeding experiments with genistein using rodent animal models to evaluate the estrogen agonistic potential of genistein.

446 HYPOTHESIZED HEALTH BENEFITS OF SOYBEAN ISOFLAVONES

M J Messina, Consultant. *Port Townsend, WA.* Sponsor: W Helferich

Soybeans are unique in that they are a rich dietary source of isoflavones. The primary soybean isoflavones are genistein and daidzein (aglycones). Daidzein and genistein are weak estrogens, possessing anywhere from approximately 1 x 10–3 to 1 x 10–5 the activity of estrogen. Soy consumption in both animals and humans has been found to lower blood cholesterol, and is associated with a reduced risk of hormone related cancers — the weak estrogen activity of genistein and daidzein has been postulated as contributing to these beneficial effects. Similarly, soy feeding has been shown to relieve menopause symptoms and to inhibit bone resorption. Consequently, soy is increasingly being viewed as a possible natural alternative to estrogen replacement therapy. However, it is the hypothesized anticancer effects of the soybean isoflavones that has really attracted the interest of the research community. Genistein has been shown to inhibit the growth of a wide range of cancer cells *in vitro*. Genistein inhibits the activity of several enzymes involved in cell growth and regulation. Human studies show that blood levels of isoflavones in response to soy consumption reach levels somewhat comparable to the concentrations of isoflavones required to inhibit the growth of cancer cells *in vitro*. Animal work, although very limited, has confirmed these *in vitro* findings. Epidemiologic studies, although inconsistent, show that in those cases where soy intake is protective, that the consumption of only one serving per day is associated with a reduced cancer risk. Overall, although still largely speculative, the data suggest that incorporating reasonable amounts of soyfoods into the diet may favorably impact public health.

447 DIETARY ESTROGENS AND ANTIESTROGENS: A QUESTION OF DOSE AND POTENCY

S Safe. Veterinary Physiology & Pharmacology. *Texas A&M Univ., College Station, TX*

It has been hypothesized that environmental estrogens may play a role in breast cancer in women and reproductive problems in men. The human diet contains an array of hormone mimics which directly or indirectly induce or inhibit estrogen-induced responses. Estrogenic bioflavoids which act through the estrogen receptor (ER) are weak estrogens and some of these compounds such as naringenin also exhibit partial antiestrogenic activity. 2, 3, 7, 8-Tectrachlorodibenzo-p-dioxin (TCDD) and related environmental contaminants which are also present in the human diet bind to the aryl hydrocarbon (Ah) receptor and inhibit a diverse spectrum of estrogen-induced responses. In addition, other classes of natural Ah receptor agonists (endodioxins) in the diet include polynuclear aromatic hydrocarbons (PAHs) and aromatic amines in cooked foods, indole-3-carbinol and related hetero-PAHs in vegetables and these compounds are also antiestrogenic. A mass balance of estrogens and antiestrogens in the human diet indicates that levels of estrogenic pesticides and industrial compounds are at lower levels than natural estrogens and antiestrogens and are therefore unlikely to be responsible for estrogen-related adverse human health effects.

448 MODULATION OF CATECHOLESTROGEN METABOLISM AND ITS ROLE IN ESTROGEN-INDUCED TUMORS

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Catecholestrogen metabolites have been postulated to mediate the induction of kidney tumors in Syrian hamsters by estradiol. To examine this hypothesis we studied the effects of a modulator of tumor incidence on carecholestrogen metabolism. The bioflavonoid quercetin, which inhibits tumors induced by chemical carcinogens, in contrast potentiated the severity of estradiol-induced kidney tumors. In hamsters treated with 50 μg estradiol, renal and hepatic concentrations of 2- and 4-hydroxyestradiol were increased by 80 and 59% and 48 and 59%, respectively, in response to quercetin. In hamsters treated with 200 μg estradiol, this flavonoid decreased the urinary excretion of 2- and 4-methoxyestradiol by 63–65%, decreased renal S-adenosylmethionine concentrations by 25% and increased S-adenosylhomocysteine concentrations by 89%. Kinetic studies in vitro demonstrated that S-adenosylhomocysteine at the prevailing concentrations in the kidney inhibited the O-methylation of catecholestrogen metabolites more effectively than did quercetin. It is concluded that quercetin increased the severity of estrogen-induced carcinogenesis by inhibiting the O-methylation of catecholestrogen metabolites, thus increasing the concentrations of these substrates for metabolic redox cycling and free radical generation. These data are consistent with a role of catecholestrogen metabolites as initiators of estrogen-induced tumors.

449 ARSENIC HYPERMETHYLATES THE *p53* PROMOTER IN HUMAN LUNG CELLS

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Arsenic is a carcinogen to which humans are exposed primarily through drinking water. The mechanism of action of arsenic as a carcinogen has not been elucidated, although the mechanisms for its action as a poison are well appreciated. We tested the hypothesis that a mechanism of arsenic carcinogenesis could involve alterations of cytosine methylation patterns in genes that are targets in arsenic cancer target tissues. Such alterations of DNA methylation patterns might occur because arsenic might compete for methyl groups as it is detoxified by methylation through an S-adenosylmethionine (SAM) / methyltransferase(s) pathway, and/or because arsenic is a potent enzyme inhibitor and may inhibit methyltransferases that utilize SAM, leading to changes in the size of methyl donor pool. These events might alter the activity of *de novo* or maintenance cytosine methyltransferase resulting in under- or over-methylation of cytosine, and result in changes in transcription and expression of tumor suppressor genes and oncogenes. We utilized bisulfite modification of DNA and DNA sequencing to visualize 5-methylcytosine (5-MeC) residues in a 350 base-pair region of the promoter of the *p53* gene in A549 cells exposed to sodium arsenite. We found that exposure 0.8 to 2 μM sodium arsenite can profoundly increase 5-MeC levels in a region of the *p53* promoter. We are currently assessing the sequence-specific distribution of 5-MeC residues in the promoter of *p53* in arsenic-exposed and control cells. We will discuss a new model for arsenic carcinogenesis where DNA methylation alterations underlie its mechanism of action. *This is an abstract of a proposed presentation and does not necessarily reflect policy of the U.S. EPA.*

450 EFFECT OF PARTIAL HEPATECTOMY ON THE MUTANT FREQUENCY AND MUTATION SPECTRUM OF *lacI* IN TRANSGENIC MICE TREATED WITH N-ETHYL-N-NITROSOUREA

B S Shane[1], V J Wood[2], M L Cunningham[2], and K R Tindall[2]. [1]*Louisiana State University, Baton Rouge, LA,* [2]*NIEHS, Research Triangle Park, NC*

The Big Blue® transgenic mouse permits the study of the role of pharmacodynamics on mutation frequency (MF) at a chromosomally integrated *lacI* target gene following *in vivo* xenobiotic exposure. We have studied the effects of cellular proliferation on MF at *lacI* in the liver of male Big Blue® mice. Eight week old mice were injected with either saline [control] or 50 mg/kg N-ethyl-N-nitrosourea (ENU) [treated] for five consecutive days (days 1–5) followed by a 40% partial or sham hepatectomy on day 6. Animals were sacrificed on days 21 and 51, corresponding to days 15 and 45 following hepatectomy. The observed MF ($9.0 \pm 1.95 \times 10^{-5}$) in control mice that were subjected to hepatectomy was not significantly different at the time of hepatectomy (day 6) compared to the MF observed at the time of sacrifice at either 21 (MF $= 1.9 \pm 0.4 \times 10^{-4}$) or 51 (MF $= 1.0 \pm 0.13 \times 10^{-4}$) days. However, hepatectomy clearly had an effect on the observed MF in the liver following ENU treatment. At sacrifice, the MF in the liver of the treated mice subjected to hepatectomy was $2.11 \pm 0.63 \times 10^{-3}$ and $2.54 \pm 0.36 \times 10^{-3}$ at 21 and 51 days, respectively. These MF values are more than one order of magnitude higher than the MF observed in the liver from the same animals taken at the time of hepatectomy on day 6 (MF $= 1.9 \pm 0.29 \times 10^{-4}$). In addition, the ENU-treated mice subjected to hepatectomy showed a substantially higher MF than was observed in treated mice without hepatectomy. At 21 days the MF in the mice that received ENU alone was $5.3 \pm 1.3 \times 10^{-4}$ while at 51 days the MF was $6.7 \pm 0.85 \times 10^{-4}$. These results suggest that cellular proliferation induced by partial hepatectomy substantially increases the frequency of ENU-induced mutations in the liver. The majority of mutations observed were single-base substitutions comprised mainly of AT>TA transversions and GC>AT transitions with a few AT>GC transitions. While the yield of these types of mutations varied between those animals that received a partial hepatetcomy and those that did not, both data sets implicate ethyl adducts at either guanine and thymine residues as mutagenic lesions in this system.

451 p53 EXPRESSION DURING ACETAMINOPHEN-INDUCED HEPATIC NECROSIS IN MICE

M B Carter[1], V M Salas[1], B Copple[2], P L Iversen[2], G B Corcoran[1]. [1]*Toxicology Program, University of New Mexico College of Pharmacy, Albuquerque, NM;* [2]*Department of Pharmacology, University of Nebraska Medical Center, Omaha, NE*

Toxic doses of the arylating drug acetaminophen (APAP) cause loss of genomic DNA at 2 hr with appearance of single and double strand DNA breaks, consistent with Ca^{2+}-endo-nuclease activation (FASEB J 7:453). p53 is increased by DNA damage, including single and double strand breaks. A nuclear cascade of events then prevents DNA-damaged cells from progressing through cell cycle by blocking cells at G_1, permitting DNA repair and reducing damage fixation. Large p53 increases have been shown to cause some cells to die by apoptosis. It is not known if p53 is increased by APAP-induced DNA damage or if p53 plays any role in necrotic cell death. Male NIH Swiss mice were treated with 500 mg/kg APAP ip and hepatic p53 protein was evaluated by western blotting with anti-p53 antibody (Oncogene Sciences) and enhanced chemiluminescence detection. Immunoreactive p53 increased slightly over control values at 2 hrs (114%) after APAP treatment, peaked at 4–6 hr (213–219%) and remained elevated above control values at 24 hr (146%). The cellular concentration of p53 protein can be acutely decreased or increased by various treatments. Such treatments will be used to evaluate the role of p53 in necrotic death in vivo and in human and murine hepatocytes (NIH ES 06345).

452 Br-A23187 TOXICITY IN HEPATOCYTES: ROLE OF THE MITOCHONDRIAL PERMEABILITY TRANSITION (MPT)

T Qian, B Herman and J J Lemasters. *Department of Cell Biology & Anatomy, University of North Carolina at Chapel Hill, Chapel Hill, NC*

Calcium has long been postulated to play a critical role in lethal cell injury. Here, we studied the toxicity of Br-A23187, a calcium ionophore, to overnight cultured rat hepatocytes. Cell viability was assessed by propidium iodide fluorometry. Mitochondrial matrix free Ca^{2+}, mitochondrial membrane potential ($\Delta\Psi$) and permeability of mitochondrial membranes were monitored with Rhod-2, tetramethylrhodamine methyl ester or rhodamine 123, and calcein, respectively, using laser scanning confocal microscopy. After exposure to 10–20 μM Br-A23187, >60% of hepatocytes lost viability within 1 hour. Loss of cell viability was preceded in sequence by an increase of mitochondrial free Ca^{2+}, collapse of mitochondrial $\Delta\Psi$, and redistribution of calcein from the cytosol into mitochondrial space, the last signifying onset of the MPT. Cyclosporin A (1 μM), which specifically inhibits onset of the MPT, delayed redistribution of calcein and cell killing, but did not prevent mitochondrial depolarization or the increase of mitochondrial free Ca^{2+}. Br-A23187-induced lethal cell injury was an ATP depletion event since fructose plus oligomycin prevented cell killing. In conclusion, Br-A23187 directly caused increase of mitochondrial Ca^{2+} uptake and mitochondrial depolarization, which in turn appeared to induce the MPT and cell killing. Cyclosporin A delayed onset of MPT and protected against cell killing, possibly by inhibiting the MPT-induced stimulation of mitochondrial ATPases.

453 OVEREXPRESSION OF HUMAN METALLOTHIONEIN II SPECIFICALLY IN THE HEART OF TRANSGENIC MICE

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Metallothionein (MT) is a low molecular weight and cysteine-rich protein. It may provide protection against free radical-induced tissue injury in biological systems. Heart has weak antioxidant systems and is vulnerable to oxidative stress. The present research was undertaken to develop a transgenic mouse model in which MT is specifically overexpressed in the heart to study the antioxidative function of MT in this organ. A transgene for overexpression of MT in the heart of transgenic mice was constructed. This transgene contained fragments from the human MT-II gene ligated behind the mouse alpha cardiac myosin heavy chain promoter. A standard procedure was used to produce transgenic mice. The transgenic founders that had integrated the transgene were identified using Southern and Dot blot assays and PCR procedure. MT concentrations and its mRNA levels in the heart and other organs were measured. These analyses revealed that we have produced 6 transgenic mouse lines in which MT is specifically overexpressed in the heart. A wide range of expression levels were observed among these transgenic lines. MT levels were not altered in other tissues including liver, lung, kidney, and skeletal muscles. This unique experimental model provides an unequivocal approach to understanding the role of MT in protection against oxidative injury to the heart. Supported in part by NIH Grant CA63752 and CA68125, and A Grant-in-Aid from the American Heart Association 94007070.

454 METALLOTHIONEIN (MT) EXPRESSION AFFECTS SPONTANEOUS AND INDUCED MUTAGENESIS

T G Rossman, E I Goncharova, and N Dolzhanskaya. *NYU Medical Center, New York, NY.* Sponsor: M Costa

Chinese hamster G12 cells (a V79 derivative with a single copy of *E. coli gpt* as a mutagenic target gene) were transfected with a vector containing the mouse MT-1 gene under control of its own promoter and MT-1 overproducing cells were isolated. G12 cells were also transfected with a vector containing Chinese hamster MT-II antisense cDNA, and antisense-expressing cells were isolated. MT-1 transfectants had decreased spontaneous mutagenesis and the antisense transfectants had elevated spontaneous mutagenesis. Modulation of MT expression showed that spontaneous mutagenesis, but not gene amplification (PALA-resistance) is inversely related to MT expression. MT-1 transfectants showed decreased cytotoxicity and mutagenesis in response to cadmium, mAMSA and other topoisomerase inhibitors which produce free radicals, 5-azacytidine, and menadion, but not to small alkylating agents. MT can block spontaneous and TPA-induced oxidative stress. A significant increase in spontaneous mutagenesis occurs when G12 cells are grown in high Zn^{2+} up to 12μM; higher concentrations (which induce MT) resulted in decreased mutagenesis compared with controls (approx. 2μM Zn^{2+}). In contrast, the low spontaneous mutation rate of MT-1 transfectants was unaffected by Zn^{2+}. Although it does not redox cycle, Zn^{2+} unopposed by MT increases oxidative stress in cells. We suggest that MT evolved to play a protective role against mutagenesis by free Zn^{2+}. (Supported by NIH grant CA 61319)

455 NeRD, A NOVEL DNA REGULATORY ELEMENT WHICH SILENCES TCDD-INDUCIBLE CYP1A1 GENE EXPRESSION IN RAT KERATINOCYTES

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Polycyclic aromatic hydrocarbons (PAHs), such as 3-methylcholanthrene are toxic to rat epidermal cells in early passages (3 to 6) but not late passage (>15). Given the role of P4501A1 in the metabolic activation of numerous PAHs, the regulation of this gene in early and late passage cells was examined. Little or no 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin)-inducible P4501A1 (or P4501B1) mRNA or enzyme activity was observed in the high passage compared to low passage cultures. Similarly, transfection of a luciferase reporter construct containing -1317 to +256 bp of the upstream region of the murine CYP1A1 gene (including four dioxin responsive elements (DREs)) was TCDD-inducible in low but not high passage cells. Ligand binding and transfection experiments with a reporter construct containing DREs upstream of a heterologous promoter revealed that early and late passage cells contain functional Ah receptor (AhR) complexes. Deletion analysis studies identified the negative regulatory DNA (NeRD) element 3′ of the DREs responsible for CYP1A1 gene silencing in late passage cells (TCDD induciblility was regained with its deletion). DNA binding analysis with late passage cell nuclear extracts revealed a specific protein(s) which bound to a NeRD oligonucleotide. Our results suggest that high passage rat epidermal cells contain a factor(s) which negatively regulates expression of CYP1A1, resulting in a loss of PAH sensitivity.

456 DETOXIFICATION OF ORGANOPHOSPHATES BY CRYOIMMOBILIZED MICROBIAL CELLS

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It has been demonstrated that cryoimmobilized recombinant *E. coli* are capable of hydrolyzing paraoxon (PO) and parathion (PS) to p-nitrophenol (PNP) and diethyl phosphoric acid. Cryoimmobilized wild-type *Pseudomonas putida* was used for subsequent degradation of PNP. Detoxification of PO and PS were carried out under three different conditions: (1) substrates were sequentially hydrolyzed by cryoimmobilized *E. coli* followed by cryoimmobilized *P. putida*, (2) substrates were simultaneously hydrolyzed by both biocatalysts, and (3) substrates were hydrolyzed by cryoimmobilized biocatalyst containing both *E. coli* and *P. putida*. The PO and PS degradation was monitored by PNP detection. The efficiency and the degradation rate were identical for the three conditions utilized and were dictated by the rate of PNP degradation. Four mM PO or PS was completely degraded to PNP within 6 hours. An increase in initial substrate concentration led to significant inhibition of PNP degradation. A positive correlation between biocatalyst quantity and the degradation rate was observed. A ratio of *E. coli* to *P. putida* of 1:100 was shown to be optimal for efficient co-cryoimmobilization. No loss of activity was detected for PO and PS hydrolysis for three consecutive cycles with both the co-cryoimmobilized biocatalyst and a mixture of the two individually immobilized cultures. The biocatalysts exhibited stable activity in the detoxification of organophosphates in both aqueous and biphasic systems.

457 INDUCTION OF AP-1 AND NF-kB TRANSCRIPTION FACTOR BINDING ACTIVITY AND UP-REGULATION OF c-JUN, c-MYC AND p53 PROTEINS IN HUMAN LEUKEMIA CELLS EXPOSED TO TRANS, TRANS-MUCONALDEHYDE, A TOXIC BENZENE METABOLITE

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Alterations of gene expression associated with exposure to the benzene metabolite trans, trans-muconaldehyde (muconaldehyde) were investigated using human HL-60 myeloid leukemia cells. There was a significantly increased level of DNA binding activity of AP-1 in cells exposed to 0.1 μM muconaldehyde for 2 hr as determined by gel retardation. Cells incubated with 1 μM muconaldehyde for 2 hr also showed an increase in NF-kB binding activity. Increased levels of binding activity were not detected for AP-1 at 1-100 uM muconaldehyde concentration or 10-100 uM for NF-kB. Increased protein synthesis of c-jun was detected in HL-60 cells treated with both 0.1 μM and 1 μM muconaldehyde for 2 hr based on immunoprecipitation. Treatment with muconaldehyde (0.1-100 μM) resulted in increased c-myc and p53 protein synthesis. The present results demonstrate that muconaldehyde affects DNA binding activities of AP-1 and NF-kB, two transcription factors known to be activated by reactive oxygen species. The latter have been implicated in the toxicity of reactive benzene metabolites. The results suggest that activation of c-jun, c-myc and p53 genes may play a role in the biological effects of muconaldehyde. This work was supported by NIEHS Grant ESO2558 and NIEHS Center Grant ESO5022.

458 GENETICALLY DETERMINED DIFFERENCES IN XENOBIOTIC METABOLISM AS A RISK FACTOR IN PARKINSON'S DISEASE

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Differences in xenobiotic metabolism can have a profound influence on individual susceptibility to diseases associated with environmental toxins. Exposure to pesticides and herbicides are reported to be associated with an increased risk for Parkinson's disease (PD). To test the hypothesis that genetic polymorphsims in xenobiotic-metabolizing enzymes may predispose individuals to PD, 144 Caucasian PD patients and 141 controls were genotyped for Glutathione *S*-Transferase M1 gene(*GSTM1*), encoding for the major Phase II conjugation enzyme in the xenobiotic-metabolizing pathway. Consistent with previous reports, about 53% of the controls were homozygous for the null *GSTM1* allele, corresponding to the defective phenotype. The frequency of homozygosity for the null *GSTM1* allele in PD is slightly but not statistically significantly higher (59%). When the analysis was stratified by the age at disease onset and sex, still no significant difference was found as compared to controls. However, risk of PD (Odds ratio 11, 95% confidence interval 1.1 – 109.7) was significantly increased in individuals with the combined genotype of homozygosity for the null *GSTM1* and *CYP2D6L*, encoding a subfamily of the major Phase I xenobiotic-metabolizing enzyme cytochrome P450. The additional effects of another Phase II enzyme, catechol *O*-methyltransferase, involved in dopamine metabolism, will also be discussed. We conclude that metabolic defects in multiple xenobiotic enzymes confer greater risk for PD and that gene-environment interaction may play an important role in causing the disease.

459 LEAD EXPOSURE ALTERS THE DRUG METABOLIC ACTIVITY AND THE HOMEOSTASIS OF ESSENTIAL METAL IONS IN LENTICULAR SYSTEM OF THE RATS

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Potential lead exposure to the eyes because of the use of traditional cosmetic, Kohl in Asia, Africa and the Middle East has been a subject of recent debate to the scientific community (Sprinkle, 1995). In continuation to our earlier work (Dwivedi, 1987) we therefore examine in present study, the drug metabolic activity and the homeostasis of essential metal ions in lenticular system of the adult rats exposed to long term low level of lead. The results of our investigation demonstrate that long term low level of lead exposure impaired the phase I & phase II metabolic activity of lenticular system when assessed by aminopyrene demethylase, benzo[a]pyrene hydroxylase, aniline hydroxylase and UDP glucuronyl transferase (UDPGT), glutathione S-transferase (GST). A more pronounced decrease (55%) in GST was noticed compared to UDPGT, aminopyrene demethylase, benzo[a]pyrene hydroxylase and aniline hydroxylase (20–30 %). Lead induced alterations were more predominant at 3 month when compared to controls untreated. Increased lead concentration in lenticular system of the rats as monitored by atomic absorption spectroscopy resulted in a significant decrease (30–40%) in the levels of Ca, Cu, Zn and Fe. Respective increase in blood lead level was also monitored parallel to increase in ocular lead concentration at different time points in lead treated rats. The present investigation therefore, provide data which suggest that lead exposure to rats results in a profound impairment in the homeostasis of essential metal ions and lenticular drug metabolizing enzymatic activity. Whether such a decrease in these functions reflect an inhibition of protein synthesis at transcriptional/post transcriptional levels or gene regulation at molecular level remains to be established.

460 DOES THE GUINEA PIG METHYLATE ARSENITE AND MMA?

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A rapid and specific *in vitro* assay utilizing radiolabeled S-adenosylmethionine (^{3}H-SAM) has been developed by this laboratory for the methylation of arsenite

to monomethylarsonate (MMA) and MMA to dimethylarsinate (DMA) by rabbit liver methyltransferases. The assay has detected the enzymes that methylate arsenic species in the rabbit, mouse, hamster, rat, and rhesus monkey. However, when guinea pig liver cytosol was incubated with ^{3}H-SAM, neither MMA nor DMA was detected. Furthermore, when guinea pigs were given [^{73}As]-arsenate (400μg/kg, 25μCi/kg) intraperitoneally very little or no methyl arsenic species were detected in the urine. Urine was collected after 12 and 24 hours and the arsenic species separated by cation exchange chromatography. At 12 hours postexposure, 98% and 1% of the arsenic recovered from the column eluted as inorganic and dimethylated arsenic respectively. No MMA was detected after 12 hours. At 24 hours postexposure, 93%, 2%, and 4% of the arsenic recovered from the column eluted as inorganic, monomethylated, and dimethylated arsenic respectively. The guinea pig, like the marmoset monkey and chimpanzee, but unlike other animals studied, does not appear to be able to biotransform inorganic arsenic to MMA and DMA. The results of this study and the biotransformation variances among other mammals suggest that the expression of these enzymes may be polymorphic. (Supported by Superfund Program NIEHS Grant #ES-04940 and NIEHS Center Grant #P30-ES-06694)

461 EFFECTS OF CHRONIC ADMINISTRATION OF SODIUM DICHROMATE [Cr(VI)] AND CADMIUM CHLORIDE [Cd(II)] TO RATS ON THE EXCRETION OF URINARY LIPID METABOLITES AND PRODUCTION OF MITOCHONDRIAL AND MICROSOMAL LIPID PEROXIDATION

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Acute administration of sodium dichromate [Cr(VI)] and cadmium chloride [Cd(II)] to laboratory animals produces oxidative damage in hepatic and brain tissues. However, a lack of information exists regarding the effects of chronic exposure to these cations. Therefore, female Sprague-Dawley rats were chronically treated daily p.o. with a low dose (0.05 LD_{50}) of sodium dichromate (2.5 mg/kg) or cadmium chloride (4.4 mg/kg) for 120 days. Urine samples were collected in the frozen state over dry ice on days 0, 15, 30, 45, 60, 75, 90, 105, and 120. Animals were killed at these same time points, and hepatic and brain subcellular fractions were prepared. Urinary lipid metabolites including malondialdehyde (MDA), formaldehyde (FA), acetaldehyde (ACT), and acetone (ACON) were identified and quantified using HPLC in conjunction with GC-MS. The urinary excretions of MDA, FA, ACT, and ACON were 2.1-, 1.8-, 2.0-, and 1.8-fold, respectively, higher than the control values 75 days post-treatment in the [Cr(VI)] treated rats, and 1.8-, 1.5-, 1.9-, and 1.4-fold, respectively, higher in the Cd(II) treated rats. Little or no significant increases in the excretions of these lipid metabolites occurred with time thereafter. Lipid peroxidation (LP) was assessed in hepatic and brain tissues. Lipid peroxidation increased 1.8-and 1.6-fold in liver and brain mitochondria, respectively, in Cr(VI) treated rats after 75 days, and 2.0- and 2.3-fold in liver and brain mitochondria, respectively, in Cd(II) treated rats at this same time point. The results clearly indicate that chronic administration of low doses of Cr(VI) and Cd(II) salts induce oxidative stress which result in tissue damaging effects.

462 AN ALUMINUM-INDUCED INCREASE IN GFAP IS ATTENUATED BY SOME 3-HYDROXYPYRIDIN-4-ONE CHELATORS

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Astrocytic hypertrophy is a generic reaction to CNS injury. The hallmark of this response is an increase in glial fibrillary acidic protein (GFAP). In this study we assessed the effects of aluminum (Al) on GFAP to determine potential sites of Al-induced brain damage. Chelators were used to reverse potential Al effects. Neuronal proteins, synaptophysin (SP), synapsin I (S-I) & neurofilament 68 (NF-68), and the myelin protein, myelin basic protein (MBP), were examined to determine cellular targets of Al-induced brain damage. Rabbits were given 20 Na or Al (100 μmoles/kg) lactate iv injections over 1 month, followed by 12 doses, over 1 month, of deferoxamine (DFO) given iv or saline or one of six 3-hydroxypyridin-4-ones (CPs) given po. Their brains were removed for protein and Al analyses. GFAP was assayed by ELISA; other proteins were assayed by PhosphorImager analysis of ^{125}I Protein A immunoblots. Frontal cortical GFAP of Al loaded-saline treated rabbits was significantly greater than in non-Al-loaded saline-treated rabbits. CP93, CP52 and CP24 significantly reduced GFAP; DFO, CP40, CP20 and CP94 did not. Frontal cortical GFAP and Al concentrations positively correlated. There were no significant changes in cortical SP, S-I, NF-68 or MBP or in hippocampal or cerebellar GFAP. These results are consistent with a report showing an Al-induced increase in cortical GFAP in guinea pigs (Exp. Neurol. 94: 519, 1986) and are suggestive of an Al-induced astrocytic response to subtle CNS damage. The results also suggest a protective role of some chelators against Al-induced neurotoxicity that may be due to reduction of brain Al. Supported by NIH ES 4640.

463 LEAD (Pb) TOXICITY AND THE CHOROID PLEXUS (CP): DEPRESSION OF CEREBROSPINAL FLUID (CSF) TRANSTHYRETIN (TTR) IN RATS FOLLOWING CHRONIC Pb EXPOSURE

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The choroid plexus (CP), which is responsible for the maintenance of the biochemical milieus of CSF, avidly sequesters Pb. In order to test the hypothesis that chronic Pb exposure may be associated with impaired CP function, we exposed weanling Sprague-Dawley rats to Pb in drinking water at doses of 0, 50 or 250 μg Pb/ml (as Pb acetate) for 30, 60 or 90 days. The function of CP was assessed as reflected by CSF concentrations of TTR (a major CSF protein manufactured by the CP), CSF essential metal ions (Ca, Mg, K, and Na) and CP protein kinase C (PKC) activity. CSF TTR contents were determined by RIA using a monospecific rabbit anti-rat TTR polyclonal antibody, and CSF metal ions analyzed by flame AAS. Two-way ANOVA of CSF TTR concentrations revealed highly significant dose, time, and dose-by-time effects (all $p<0.009$). Moreover, the % of reduction of CSF TTR was directly correlated with CP Pb concentrations ($r=0.703$, $p<0.05$). Pb exposure did not markedly altered CSF concentrations of essential metal ions, nor did it modulate CP PKC activities in vivo. Since TTR is responsible for transport of thyroid hormones to the developing brain, we speculate that the depression of CP TTR production (and/or secretion) by Pb may impair brain development in young animals by depriving the CNS of thyroid hormones. (Supported in part by Grants P20-ES06831, RO1-ES03460, and the Lucille Markey Foundation)

464 EFFECT OF SELENIUM DEFICIENCY ON THE INCIDENCE OF LUNG TUMOR IN A/J MICE

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As tumor generation is thought to be accelerated by oxidative stress, modulation of oxidative stress is expected to affect tumor incidence in human and animals. Since selenium constitutes an active center of glutathione peroxidase which protects tissues from oxidative injury, its preventive role against carcinogenesis has been an attractive research subject for many investigators. Though there are many papers reporting that relatively high dose of selenium suppressed carcinogenicity of chemicals, only a limited number of papers dealt with the relationship between selenium deficiency and spontaneous tumor incidence. Recently the suppression of spontaneous lung tumor generation in A/J mice by vitamin E was reported, prompting us to examine whether the selenium deficiency enhances the lung tumor incidence in A/J mice. Female A/J mice (4 week old) were fed selenium deficient or selenium adequate (0.4 ppm Se as sodium selenite) diet for 38 weeks, and the number of nodules in lung was counted. The tumor incidence was 7% (1/15) and 25%(2/8) in Se(−) and Se(+) mice, respectively. Average number of nodules in Se(−) lung (0.07) was less than Se(+) (0.57). These results indicate that selenium deficiency does not enhance spontaneous lung tumor generation in A/J mice. Together with our recent finding that selenium compounds can be a source for active oxygen species, these data may suggest that selenium can act either as a protective or as a causative factor in carcinogenesis.

465 DEVELOPMENT OF CHEMICAL SPECIFIC PROTEINURIA PATTERNS IN HAMSTERS FOLLOWING IN VIVO EXPOSURE TO GaAs AND InAs PARTICLES

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Subcutaneous administration of 100 mg/kg GaAs and InAs particles to hamsters produced marked increases in the expression of a number of major stress protein families in renal proximal tubule cells as monitored by ^{35}S-labeling, 2-dimensional gel electrophoresis and Western blot analysis.. These

effects were associated with the development of GaAs and InAs-specific low molecular weight proteinuria patterns as monitored by 2-dimensional gel electrophoresis and silver staining. These data suggest that stress proteins could "chaperone" damaged proteins from renal tubule cells into the urine. Alternatively, the low molecular weight proteinuria could be secondary to decreased tubular reabsorption of filtered proteins. In either case, the observed proteinuria patterns may be potentially useful as biomarkers of GaAs or InAs effects on renal tubule cells prior to overt renal damage. Supported in part by NIH grant ESO 4979.

466 INDUCTION OF KIDNEY HEME OXYGENASE BY INDIUM, GALLIUM, AND ARSENIC

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Indium (In), gallium (Ga) and arsenic (As) are major components of III-V semiconductors. The binary compounds InAs and GaAs possess distinct advantages in electronic speeds over silicon-based semi-conductors; hence increased human exposure is expected. In, Ga, and As alone or in combination are known to exert toxic effects on various tissues, including the kidney. Previous studies have shown that marked alterations in protein synthesis patterns occur in hamster kidneys following *in vivo* exposure to As; included in these changes is the induction of a 32-kDa stress protein thought to be heme oxygenase (HO-1). The objectives of this study were to establish the identity of the 32-kDa protein as HO-1 and to test the hypotheses that 1) In and Ga may alter the effects of As in the kidney and 2) HO-1 is induced by damage from oxidative intermediates generated by the metal ions. Supernatants obtained from kidney proximal cortical tubule cells exposed *in vitro* and from rat kidneys exposed *in vivo* to In, Ga, and As alone and in combination were assayed for HO-1 by Western blot analysis. HO-1 was increased in both the *in vitro* and *in vivo* As, In and Ga exposed cells. In and Ga when administered with As did not alter the HO-1 response. The capacity of the metals to generate oxidative radicals was determined by carbon monoxide (CO) and thiobarbituric acid reactive substances (TBARS) measurements in an *in vitro* system. All three metals had little capacity to directly generate oxidative products; furthermore, studies showed that In and Ga inhibited the production of CO and TBARS when added to an Fe^{+3}/ascorbic acid free radical generating system. Results of these studies indicate that 1) In and Ga do not alter the stress protein response of kidney cells exposed to As and 2) In, Ga and As appear to induce HO-1 by methods other than the direct production of oxidative free radicals. Supported in part by NIH ESO 4979.

467 DEVELOPMENTAL DELAY AND PARTIAL DEMASCULINIZATION IN MALE RATS TREATED WITH LEAD IN A LIFETIME EXPOSURE MODEL

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Development lead exposure is well known to produce reproductive toxicity and growth effects in humans and experimental animals. However, the molecular mechanisms underlying these phenomena remain to be elucidated. In the current study, time-impregnated female Sprague-Dawley rats (N = 9–11 / group) were exposed *ad libitum* to lead acetate in the drinking water at doses from 0–0.6 % beginning on gestational d 5. Exposure of dams and their pups (culled to 4 male and 4 female animals/litter at birth) was continued until sacrifice. At 0, 0.05%, 0.15% and 0.45% lead acetate, one male and one female pup/litter were sacrificed at age 21,35,55 and 85 d. At 0.6% lead acetate all pups were sacrificed at age 85 d. Animals were weighed every 3–5 days and at sacrifice male secondary sex organs were dissected and weighed. Plasma from male pups was prepared and assayed for testosterone by radioimmunoassay. Livers were collected and subcellular fractions prepared by differential ultracentrifugation. Sexually dimorphic metabolism of testosterone and cytochrome P450 isozyme expression was examined in microsomes. Sexually dimorphic sulfotransferase activity and expression were examined in cytosol. At lead exposures up to 0.45%, a significant ($p < 0.05$) dose-responsive delay was observed in development of the prostate gland, male specific cytochrome P450 CYP 2C11-expression and it's associated testosterone 2α-hydroxylase activity and male specific N-hydroxy-2 acetylaminofluorine (N-OH-2AAF) sulfotransferase accompanied by a significant ($p < 0.05$) dose responsive decrease in male pubertal growth rate and pubertal serum testosterone levels. However, most of these parameters returned to control levels by age 85 d inspite of continued lead exposure. Only the highest lifetime exposure to 0.6% lead acetate, resulted in significant suppression of serum testosterone in adult male animals. This was accompanied by significant ($p < 0.05$) decreases in male specific N-OH-2AAF and estrogen sulfotransferase activities but by only an insignificant 10% decrease in expression of male specific CYP 2C11. Supported in part by RO1 ES06684 to M.J.J.R. and the S.R. Noble Foundation (D.P.R.).

468 EFFECTS OF TRIETHYL LEAD ADMINISTRATION ON THE EXPRESSION OF GLUTATHIONE S-TRANSFERASE ISOENZYMES AND NAD(P)H:QUINONE OXIDOREDUCTASE IN RAT KIDNEY AND LIVER

D A Daggett, E F Nuwaysir, S A Schroeder, L S Wright, S Kornguth, and F L Siegel. *(1995). Environmental Toxicology Center, University of Wisconsin, Madison, WI.* Sponsor: C Jefcoate

The effects of triethyl lead (TEL) on expression of glutathione S-transferases (GSTs) and NAD(P)H:quinone oxidoreductase (QR) in rat liver and kidney were determined. Fischer 344 rats were given one ip injection of TEL and GST activity, GST isoenzyme levels, mRNA levels of alpha class GST subunits Ya_1, Ya_2, and Yc_1 and activity of NAD(P)H:quinone oxidoreductase (QR) were determined. Treatment of rats with TEL caused an increase in GST activity in kidney; GST subunits Yb_1, Yp, Yc_1, Ya_1, Ya_2, and Yk were also significantly elevated. The largest increase was a 3.2-fold increase in subunit Yb_1. The levels of GST Ya_1, Ya_2, and Yc_1 mRNA in kidney also increased significantly. In liver, TEL significantly decreased GST activity; the level of hepatic GST subunits Yb_2, Yp, Yb_3, Ya_1, Ya_2, and Yk were similarly decreased. In contrast, liver GST Yc continued to increase from day 4 through day 14 after exposure to TEL. The mRNA levels from the alpha class GST genes Ya_1, Ya_2, and Yc_1 were reduced 12 hours after injection of TEL in liver. By 24 hours after TEL injection, Ya_1 and Ya_2 mRNA returned to basal level while Yc message increased to a level higher than controls. The activity of QR was elevated 1.5-fold in kidney and 2.7-fold in liver 4 days and 14 days after ip injection of TEL.

469 EFFECTS OF CONTINUOUS TESTOSTERONE EXPOSURE ON THE ONCOGENIC POTENTIAL OF CADMIUM IN THE MALE FISCHER RAT

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In the rodent testes, cadmium (Cd) induces severe necrosis followed by chronic degeneration. Cd is also an effective testicular tumorigen, and a single dose produces a high incidence of Leydig cell (LC) tumors. The mechanism of tumor formation is unknown, but pituitary feed-back, i.e., increased luteinizing hormone production due to low circulating androgen, has been implicated in causation of proliferative lesions within degenerate, hypofunctioning testes. Thus, the effects of androgen replacement on the testicular toxicity of Cd in Fischer (F344) rats was studied. Groups (n = 50) of 10-week-old rats either received testosterone (T) implants that approximate normal circulating levels in castrated rats or were left untreated. After 2 weeks of stabilization, rats were either given 20 μmol $CdCl_2$/kg, sc, weekly for the next 5 weeks (total dose 100 μmol/kg) or saline for a total of four treatment groups (control, T alone, T + Cd or Cd alone). Portions of each groups were killed either 10 weeks after initiation of Cd exposure (n = 10), for assessment of endocrine function, or over the next 2 years (n = 40), for assessment of testicular neoplastic lesions. At 10 weeks, Cd reduced circulating T in non-implanted rats by nearly 80%, as determined by RIA, and induced a marked weight loss of the testes (> 70%) and sex accessory glands (SAGs; reflected in a 50% reduction in prostate mass). T implantation restored circulating T levels in Cd-treated rats and prevented Cd-induced weight loss of SAGs but not of the testes. Over 2 years, Cd alone induced a > 84% incidence of LC neoplasia and a > 97% incidence of chronic degeneration, both significant increases over control rates (60% and 0%, respectively). T implantation abolished Cd-induction of LC tumors but had no effect on chronic testicular degeneration. Thus Cd-induced hypofunction of the testes, and subsequent loss of circulating T, appears to be a critical aspect in Cd induction of tumors in the rat testes.

470 IMMUNOHISTOCHEMICAL (IHC) LOCALIZATION OF HEAT SHOCK PROTEIN-72 (hsp72) IN RAT KIDNEY AFTER EXPOSURE TO MERCURY

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The hsp response evolved as a cellular strategy in order to protect and repair other proteins. Our previous studies demonstrated that the *de novo* synthesis of specific gene products, the hsps, after exposure to various toxicants is target organ-specific, i.e., mercury, a nephrotoxicant, enhanced hsp synthesis in rat kidney, but not in liver. The objective of this study was to assess the localization of hsp72 within the kidney in order to determine if the hsp response is target cell-specific. Male SD rats were injected with $HgCl_2$ (1 mg Hg/kg, iv). At 2, 4, 8, 16, or 24 hr after exposure, kidneys were removed and processed for Western blot, histopathologic, and IHC analyses. Western blot analysis demonstrated accumulation of hsp72 in kidney as early as 8 hr after exposure, with peak levels evident at 16 hr. At 4 hr after exposure, histopathologic lesions included cytoplasmic condensation, nuclear pyknosis, and detachment of the epithelium which lines the straight part of the proximal tubule in the inner cortex and outer stripe of the medulla. Over the next 20 hr, these degenerative changes progressed to frank necrosis and sloughing of epithelial casts into the tubular lumen. These lesions also extended to the proximal convoluted tubular (PCT) cells more centrifugal in the cortex, but spared the paraglomerular distal convoluted tubular (DCT) cells. IHC experiments using a hsp72 monoclonal antibody demonstrated that hsp72 accumulation occurs primarily in undamaged DCT epithelium, with less accumulation in residual undamaged PCT epithelium. No hsp72 was observed in damaged PCT cells. The data support the hypothesis that hsps play an integral role in cellular defense mechanisms. Conversely, renal tubular epithelial cells that do not express hsp72 may be more susceptible to Hg toxicity.

471 DICHLOROMETHANE METABOLISM TO FORMALDEHYDE IN RODENTS AND IN HUMANS WITH AND WITHOUT FUNCTIONAL GSTT1 AND -M1 GENES

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Metabolism of dichloromethane (DCM) to formaldehyde via a glutathione-S-transferase (GST) pathway is thought to be necessary for its carcinogenic effects in B6C3F1 mice. Since formaldehyde can bind covalently to macromolecules, we determined the quantity of DNA-protein cross-links (DPX) and RNA adducts (RFA) formed in rodent and human hepatocytes incubated with DCM or with formaldehyde. Only mouse hepatocytes formed detectable amounts of DPX from DCM. Hepatocytes from all rodent species tested formed RFA. Rates of RFA production in hepatocytes of B6C3F1 mice, F344 rats and Syrian golden hamsters were 13.7 ± 1.7, 3.7 ± 0.5, and 0.93 ± 0.09 pmol/mg RNA/hr/mM, respectively. Mouse hepatocytes incubated with formaldehyde also formed DPX and RFA. The DPX/RFA ratio in mouse hepatocytes was about 1/8 when formaldehyde was generated internally from DCM by metabolism but was about 1 when formaldehyde was added to the medium. To compare rates of human metabolism with those of rodents, the same assays were applied to three samples of human hepatocytes. In humans, GSTT1 and -M1 are polymorphic, the -T1 (Theta) form catalyzing the conjugation of halomethanes with glutathione. Two human hepatocyte samples had functional alleles for both GST genes, while one sample lacked both alleles. Only samples with functional alleles metabolized DCM to formaldehyde. Rates of RFA production were 3.7 and 1.7; DPX were undetectable. Thus, human hepatocytes possessing GST-T1 and -M1 can convert DCM to formaldehyde which binds to RNA, but this does not necessarily imply a reaction with DNA. Human hepatocytes lacking both alleles cannot convert DCM to formaldehyde and cannot form adducts with DNA or RNA.

472 POLYMORPHISM OF GLUTATHIONE TRANSFERASE THETA (GSTT1) AND ITS CONSEQUENCES FOR RISK EVALUATION OF METHYLENE CHLORIDE

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The glutathione-S-transferase (GST) pathway is held responsible for the induction of lung and liver tumors in B6C3F1 mice by methylene chloride. Tumor risk for humans occupationally exposed to methylene chloride has been considered negligible. However, a hitherto unknown polymorphic GST activity, GST theta (GSTT1), has recently been discovered. In our experiments, the activity of GST theta towards methylene chloride was investigated in human hemolysate from 13 volunteers in vitro. GSTT1 deficient individuals showed no production of formaldehyde from methylene chloride. In hemolysate of GSTT1 positive persons, the metabolism of dichloromethane in vitro can be described by Michaelis-Menten kinetics. For an individual with high GSTT1-1 enzyme activity, the maximum velocity of formaldehyde production was calculated to be 180 pmol/min per mg Hb, the K_M being approximately 60 mM methylene chloride. The distribution between GSTT1 positive and negative individuals varies widely between different ethnic populations. The possibility of a human high risk subpopulation should be considered in future risk assessment of methylene chloride.

473 DNA-PROTEIN CROSS-LINKS AND CELL PROLIFERATION IN MICE EXPOSED TO DICHLOROMETHANE: RISK ASSESSMENT WITH DPX AS DOSIMETER

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Dichloromethane (DCM) is metabolized via a glutathione-S-transferase pathway to formaldehyde, which forms DNA-protein cross-links (DPX) in mouse liver. The formation of DPX in the livers of male B6C3F1 mice exposed to [^{14}C]dichloromethane (DCM) was determined at concentrations from 500 to 4000 ppm, and the metabolic (one-carbon) incorporation of ^{14}C into DNA was measured as an index of cell proliferation in mouse lung and liver. DPX data were analyzed using an extended physiologically-based pharmacokinetic (PBPK) model. DPX formation was nonlinearly related to both the airborne concentration of DCM and the PBPK model-estimated area under the curve for DCM in mouse liver. In addition, mice exposed to DCM for 3 days (6 hr/day) at concentrations from 1500 to 4000 ppm showed increased rates of DNA synthesis in the lung indicating cell proliferation, but no increase was detected at concentrations ≤ 500 ppm, implying a no-effect level for DCM-induced cytolethality. The DPX formed in mouse liver were used as an internal measure of exposure for cancer risk assessment based on the linearized multistage (LMS) model. Maximum likelihood estimates of risk were essentially unchanged relative to estimates based on the external (airborne concentration) measure of exposure, but the upper 95% confidence limits were 3 orders-of-magnitude smaller when the internal rather than the external dosimeter was used. The improved precision resulted from a decrease in the number of terms in the probability function. The results demonstrate that an internal dosimeter such as DPX can reduce the statistical uncertainty of low-dose risk estimates calculated with the LMS model, while having only a minor effect on the maximum likelihood estimates.

474 USE OF A DUAL METHOD DOSE-ESTIMATING EXPOSURE MODEL TO ASSESS INTERNAL DOSES FROM METHYLENE CHLORIDE

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Toxicologically relevant doses are dependent on complex physiologic, biochemical, and thermodynamic regulated processes. The impact of route of exposure is just one of many complications that are difficult to assess using default approaches. Multi-compartment physiologically based pharmacokinetic (PBPK) models are better able to account for the numerous entities that affect the disposition of compunds within the body. PBPK models have been mostly used to help extrapolate between species and between dose levels. In this case we present a PBPK model, that because of extensive formulation is able to simultaneously simulate many different exposure inputs that are relevant to both experimental animals and humans. The model is capable of simulating, for example, nine different repeating inhalation exposures. Similarly, it can simulate for many gastrointestinal, intravenous, and other exposures. In addition this model is capable, for certain exposure and pharmacokinetic conditions, to calculate area under the curve measures of dose (commonly used in risk assessments) via analytical means. In this manner the model is able to compare the results from sometimes inexact numeric methods with more accurately and scientifically revealing analytical methods. This serves as both an internal quality control, as well as providing a more in-depth intuitive insight into physiologically relevant processes. The utility of such dual methods can be especially demonstrated when there are multiple competing metabolic pathways such as with methylene chloride.

475 METHYLENE CHLORIDE INDUCED MUTATIONS AT THE HPRT LOCUS OF CHO CELLS: IDENTIFICATION OF THE METABOLITE RESPONSIBLE

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Methylene chloride is carcinogenic in the mouse liver and lung by a mechanism which is believed to involve DNA single strand breaks. The basis of this DNA damage has now been investigated in CHO cells where methylene chloride activated by mouse liver S100 fraction caused DNA single strand breaks and mutations at the HPRT locus. DNA-protein crosslinking could not be detected in CHO cells exposed to methylene chloride suggesting that formaldehyde, a metabolite of methylene chloride is not the major cause of the mutations and strand breaks seen in CHO cells. The mutations at the HPRT locus, which are believed to lead to the DNA single strand breaks, have been sequenced and compared to mutations induced by 1,2-dibromoethane (1,2-DBE) and formaldehyde. Formaldehyde is a metabolite of methylene chloride and 1,2-DBE is activated by GSH conjugation in a manner analogous to methylene chloride. All three chemicals induced point mutations with a small number of insertion and deletion events. The most common mutations with both methylene chloride and 1,2-DBE were GC →AT transitions, suggesting that the glutathione conjugate of methylene chloride is responsible. Formaldehyde induced mutations were all single base transversions, mainly at AT base pairs, suggesting that formaldehyde does not contribute significantly to the mutagenicity of methylene chloride in this system. It is concluded that the genotoxicity detected as DNA single strand breaks in mouse liver and lung in vivo is probably initiated by mutations caused by the glutathione conjugate of methylene chloride.

476 METHYLENE CHLORIDE: HUMAN SAFETY ASSESSMENT

T Green. *Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK.* Sponsor: J B Knaak

B6C3F1 mice exposed to high dose levels of methylene chloride for 2 years had elevated incidences of lung and liver tumours which were not seen in rats or hamsters similarly exposed. A series of studies have examined the effects of methylene chloride on the target tissues in the mouse; compared its metabolism and pharmacokinetics in the species of interest including humans; identified its mechanism of action in mice, and determined the identity and distribution of the enzymes involved in the activation of methylene chloride. These studies have revealed a series of responses which are unique to the B6C3F1 mouse and have their origins in the nature and distribution of the GST enzymes responsible for the activation of methylene chloride. The metabolic pathway catalysed by these enzymes is at least an order of magnitude more active in mice than in the other species studied and the primary enzyme involved is localised in specific cells and the nuclei of mouse tissues, but not rat or human tissues. As a direct result of this high and localised enzyme activity, metabolites of the GST pathway cause DNA mutations and strand breaks which are believed to lead to the tumours seen in mice. In addition, methylene chloride causes cellular damage and increased cell division in the lung, and an increase in liver/bodyweight ratios in the mouse. None of the responses seen in mice can be reproduced in rats *in vivo*, or in hamster or human tissues *in vitro*, thus providing evidence that the B6C3F1 mouse is unique in its response to methylene chloride and should not be used as the basis of a human safety assessment.

477 THE ISOLATION AND DISTRIBUTION OF TWO MOUSE THETA CLASS GLUTATHIONE-S-TRANSFERASE ENZYMES ACTIVE WITH METHYLENE CHLORIDE

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The carcinogenicity of methylene chloride to mice has been attributed to metabolites of a glutathione-S-transferases (GST's) pathway which is > 10-fold more active in mice than other species, including humans. Two theta class enzymes have been isolated from B6C3F1 mice using methylene chloride as the substrate. The enzymes were not retained by glutathione affinity columns, and have apparent molecular weights of 25400 and 26100. Their amino acid and nucleotide sequences have been determined and shown to have close homology to the rat theta enzymes 12–12 and 5–5 and to human enzymes T1 and T2. GST5-5 was found to be the major enzyme involved in methylene chloride metabolism. The distribution of the enzymes has been studied in lung and liver tissues from mice, rats and humans by Northern blotting using probes generated from the coding regions of cDNA's obtained from rat, mouse and human enzymes. In situ hybridisation experiments using oligonucleotide probes of between 20 and 50 nucleotides found very high concentrations of GST 5–5 in mouse liver, specifically in cells surrounding the central vein and bile ducts, and in nuclei throughout the parenchyma. In mouse lung, GST 5–5 was concentrated in Clara and type II cells. In rat and human tissues GST 5–5 was not localised either in specific cells or in nuclei. The high concentrations and specific localisation of this enzyme in mouse liver and lung are believed to be the major contributory factor in the development of lung and liver tumours in this species.

478 DEPARTURE FROM DEFAULTS IN RISK ASSESSMENT: DOES METHYLENE CHLORIDE MEET EPA'S CRITERIA?

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The National Research Council's 1994 report, *Science and Judgment in Risk Assessment*, reviewed EPA's current practice of carcinogen risk assessment and made numerous recommendations for improvement. Among the most important are those relating to EPA's use of defaults. The report recommended that EPA should continue to regard the use of default options as a reasonable way to cope with uncertainty about the choice of appropriate models or theory and that EPA should consider attempting to give greater formality to its criteria for a departure. Research being presented by Dr. T. Green and colleagues at this meeting indicates that the mechanism of action by which methylene chloride induces the lung and liver tumors observed in B6C3F1 mice does not extrapolate to humans. The research involves methylene chloride metabolites in the glutathione-S-transferase pathway, the key role of certain mouse isoenzymes in creating large quantities of these metabolites, and studies of their genotoxicity in rodent and human cell lines. As EPA and other regulatory agencies evaluate the new data for methylene chloride, key judgments will be required on whether these data are sufficient to provide a basis for departure from the default of basing human risk estimates on the mouse bioassay data. Each step in the reasoning that the mouse response in both liver and lung is atypical of human response must be clearly established. Polymorphism in human liver enzymes requires investigation to assure that no subset of the human population will respond to methylene chloride in a similar fashion to the B6C3F1 mouse. The process used by EPA in determining to depart from the default for male rat kidney tumors involving the alpha-2u-microglobulin mechanism provides one basis for comparison.

479 THE POSSIBLE ROLE OF *S*-CHLOROMETHYLGUTATHIONE IN THE GENOTOXICITY OF DICHLOROMETHANE

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Dichloromethane, a widely used industrial solvent, is tumorigenic in liver and lungs of B6C3F$_1$ mice and its tumorigenicity is associated with glutathione-dependent bioactivation. Biotransformation of dichloromethane by glutathione S-transferases generates formaldehyde via an intermediate reactive glutathione conjugate, *S*-(chloromethyl)glutathione. The objective of the present studies was to investigate which of these metabolites is mainly responsible for the mutagenicity of dichloromethane in bacteria and to demonstrate DNA adduct formation by this pathway. Distinction between formaldehyde and *S*-(chloromethyl)glutathione as ultimate reactive intermediates formed from dichloromethane succeeded in *Salmonella typhimurium* TA 100 by using semicarbazide as a trapping agent of formaldehyde. Formaldehyde mutagenicity was blocked by an excess of semicarbazide. In contrast, mutagenicity of dichloromethane was not changed by semicarbazide suggesting the reactive glutathione conjugate as a mutating agent. Incubations of synthetic *S*-(chloromethyl)-glutathione with deoxyguanosine resulted in *S*-[1-(N^2-deoxyguanosinyl)-methyl]glutathione. This compound was not formed from formaldehyde, glutathione and deoxyguanosine. Formation of this compound was also indicated by electrospray mass spectrometry in the synthetic oligonucleotide d(GpCpGpCpGpCp) treated with *S*-(chloromethyl)glutathione and *S*-(fluoromethyl)glutathione. Similar fragmentation patterns were seen with the synthetic oligonucleotide when treated with formaldehyde, glutathione and acid, conditions resulting in the formation of *S*-[1-(N^2-deoxyguanosinyl)-methyl]-glutathione in the oligonucleotide. These results give indirect evidence for the formation of glutathione derived ^{DNA} adducts in the biotransformation of dichloromethane.

480 METHYLENE CHLORIDE: "A SPECIES SPECIFIC GENOTOXIN"

C Coutts, R Graves, G Mainwaring and T Green. *Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK.* Sponsor: J B Knaak

Methylene chloride induces lung and liver tumours in mice but not in rats and hamsters. It is metabolised by theta class GST enzymes to a reactive and potentially mutagenic, but highly unstable glutathione conjugate. Formaldehyde, a known mutagen and rat nasal carcinogen is also formed from the same pathway. The potential of these metabolites to act as genotoxins has been investigated in bacteria, CHO cells, rat, mouse, hamster and human hepatocytes and in rats and mice in vivo. The bacterial mutagenicity of methylene chloride is caused by both the GSH conjugate (in Salmonella typhimurium) and formaldehyde (in E. coli). In mouse hepatocytes and lung Clara cells, both in vitro and in vivo, methylene chloride caused DNA single strand breaks and DNA-protein crosslinking. The strand breaks were caused by the glutathione conjugate of methylene chloride and the crosslinking by formaldehyde. From the experiments comparing rats, mice, hamsters and humans, genotoxicity was confined to mouse lung and liver tissues. Human hepatocytes were completely resistant to the DNA damaging effects of methylene chloride, even at cytotoxic concentrations. The specificity for the mouse results from the very high GST activity in this species and the localisation of that activity within nuclei and specific cells of mouse liver and lung. These results, which support the concept of genotoxicity being specific to the B6C3F1 mouse, are also consistent with the large number of negative results in short-term mutagenicity tests which do not use mouse tissues, and with the outcome of the 2 year cancer studies.

481 MORTALITY OF WORKERS EMPLOYED AT A PLANT PRODUCING CELLULOSE TRIACETATE FILM BASE

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Mortality was studied among 1785 employees of a cellulose triacetate film base production facility at Brantham in the UK. The study population consisted of all male employees with a record of employment at the film factory in the period 1946–1988. The purpose of the study was to investigate patterns of mortality after exposure to methylene chloride; in particular, mortality from liver and biliary tract cancer, lung cancer, pancreatic cancer and cardiovascular disease. A total of 1473 subjects worked in jobs that entailed exposure to methylene chloride. The average duration of exposure was nine years at 19 ppm (8 hr time weighted average). In the cohort, 333 deaths were identified through 31.12.94. Mortality rates for the cohort were compared with national and local rates and expressed as Standardised Mortality Ratios (SMR). In the subcohort of workers exposed to methylene chloride, substantially reduced mortality compared to national and local rates was observed for all causes, all cancers, and the principal cancer sites of interest. Compared to national rates, mortality due to ischaemic heart disease was also reduced in this subcohort (SMR 0.92) but was slightly elevated compared to the local mortality rates, and higher than that of unexposed workers (SMR 0.74). A stronger 'healthy worker' effect had been expected, especially given a ban on smoking at the workplace. However, ischaemic heart disease mortality was lower in active employees (SMR 0.83) where a direct effect of methylene chloride exposure should be concentrated, and no in-service mortality due to ischaemic heart disease was seen in workers with the highest cumulative exposure (>800 ppm years). The results of the study provided no indication that employment at the plant, or exposure to methylene chloride, had adversely affected the mortality experience of workers.

482 MORTALITY ASSESSMENT OF TWO COHORTS OF EMPLOYEES WITH EXPOSURE TO METHYLENE CHLORIDE

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The mortality experience of two overlapping cohorts of photographic film base manufacturing employees with exposure principally to methylene chloride (MC) was assessed through 1994. In the first analysis, we updated mortality in a 1964–1970 cohort of 1013 men an additional six years. In the second study, we assessed causes of death among 1311 men initially employed between 1946 (when MC was first used) and 1970. Average 8-hr TWA exposures by job, based on extensive personal sampling, ranged to a maximum of 114 ppm in the first study group and 300 ppm in the second. Median follow-up from first exposure was greater than 35 years. Mortality for both cohorts was below expectation for all causes of death, ischemic heart disease, and total malignancies, including the lung and liver, which were target organs in the National Toxicology Program studies of mice. No significant increases were observed for any cause of death or for dose-response relationships based on either career exposure or time from first exposure. These findings were also compared with those from occupational studies of employees at two U.S. textile plants that used MC in the manufacture of cellulose triacetate fibers. The four studies combined describe the mortality experience of more than 5800 employees, including nearly 1700 women. A summary of the evidence showed (1) consistent observed-expected deficits for total deaths, all cancers, including lung and liver malignancies, and cardiovascular diseases, and (2) no evidence of a common pattern of increased mortality for any cause of death. The epidemiologic evidence is consistent with results from metabolic and pharmacokinetic studies, which indicate that the B6C3F1 mouse is unique in its response to MC and is an inappropriate model for estimating human risk.

483 THE IMMUNOLOGIC RESPONSE TO PHENYL ISOCYANATE INDICATES A POTENT CHEMICAL SENSITIZER

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Diisocyanates, as a chemical class, have been recognized for their ability to cause respiratory and dermal hypersensitivity. Toluene diisocyanate (TDI) is currently the most prevalent cause of occupational asthma. Monofunctional isocyanates have rarely been associated with hypersensitivity or asthma, although the irritant nature of these chemicals has been recognized. Phenyl isocyanate (PI), an aromatic monoisocyanate, has been reported to cause an asthma-like syndrome in rats. We sought to investigate the immunologic potency of PI, since it is a trace component in a widely used commercial diphenyl methane diisocyanate (MDI) product. The mouse ear swelling test (MEST) was employed to assess contact sensitization potential. When compared with other isocyanates, PI was found to be the most potent. The SD_{50} (the dose, in mmole/kg, predicted to sensitize 50% of the animals) values were: PI, 0.0002; hexamethylene diisocyanate, 0.0005; MDI, 0.0021; and TDI, 0.0304. The humoral immune response to PI was compared with that induced by TDI, the isocyanate which to date has produced the strongest antibody response in mice. The mean hapten-specific IgG antibody titer to PI (1.4×10^4) was significantly higher than the hapten-specific response obtained to TDI (1.3×10^3). Similarly, the hapten-specific IgG_1, titer (1.2×10^4) to PI was significantly greater than the response to TDI (6.4×10^2). Hapten-specific IgE was not detected to either isocyanate, and total serum IgE was unaffected by the isocyanate exposures. These results indicate that phenyl isocyanate is a very potent stimulator of both cellular and humoral immune responses. The mechanism(s) underlying the potencies are being studied. Supported by NIEHS 05651.

484 DETERMINATION OF INDUCED ANTIBODIES IN THE MURINE AURICULAR LYMPH NODE ASSAY AS A SENSITIVE SCREENING METHOD FOR THE ALLERGENIC AND IRRITATING POTENTIAL OF CHEMICALS FOLLOWING SKIN CONTACT

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The local auricular lymph node assay (ALN-A) has been developed as an alternative screening method for contact allergy. It offers an objective endpoint and overcomes several drawbacks of the guinea pig tests.
Initial reports on the ALN-A showed good correlation between induced proliferation and allergenicity of the compounds tested. However, more recent data have demonstrated irritant-induced proliferation as well.

We are investigating proliferative responses in concert with antibody formation in the ALN following topical application of chemical allergens and irritants. Both contact and respiratory allergens (dinitrochlorobenzene and trimellitic anhydride respectively) induced clear proliferative responses and remarkable antibody production, especially IgG1. Moreover, dose-response studies showed that IgG1 was the most sensitive isotype induced, since low-dose allergen application resulted in increase of this isotype only.

Chemical irritants, among others croton oil and tributyltin oxide, showed marked proliferative responses, but few, if any antibody production. Applica-

tion of the tolerogen dichloronitrobenzene resulted in neither lymph node enlargement nor antibody production.

Our data demonstrate that in the ALN-A proliferation does not distinguish between irritants and allergens, and we suggest that antibody production might be a reliable and sensitive parameter. Moreover, combining the parameters studied, may result in identification of chemical allergens and irritants in one assay.

485 STRUCTURE-ACTIVITY RELATIONSHIP BETWEEN CHEMICAL ALLERGENS AND CONTACT SENSITIVITY

O T Macina, J A Kramarik, and M H Karol. *Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA*

Monofunctional isocyanates have been recognized as irritant in nature and rarely associated with hypersensitivity or asthma. Phenyl isocyanate (PI), an aromatic monoisocyanate, has been reported to cause an asthma-like syndrome in rats. We investigated the immunologic potential of PI since it is a trace component in a widely used commercial diphenyl methane diisocyanate (MDI) product. Our results indicate that PI is a very strong stimulator of both cellular and humoral immune components, being more potent than toluene diisocyanate (TDI), currently the most prevalent cause of occupational asthma. We sought to investigate the mechanism(s) underlying the potency of this chemical and hypothesized that electrophilicity of the isocyanate functionality was a prime factor influencing the immunologic activity of the chemical. Using the Mouse Ear Swelling Test (MEST) as a measure of contact sensitivity, we compared the activity of a series of *para* substituted aromatic isocyanates. BALB/c mice were exposed epicutaneously to PI, nitrophenyl isocyanate (NPI), and tolyl isocyanate (TI). Five days later, mice were challenged by topical application of a non-irritating dose of the appropriate chemical, or vehicle, onto the ear. Ear thickness was measured 24 hours later. The SD_{50} (the dose, in mmole/kg, predicted to sensitize 50% of the animals) was calculated. The SD_{50} values were: PI, 0.0002; NPI, < 0.0001; TI, 0.012; and from previous studies, MDI, 0.0021; and TDI, 0.0304. Initial analysis indicates a relationship between the σ_p of the *para* substituent and the SD_{50}, supporting the role of electrophilicity in this sensitization process. Additional chemicals are being analyzed to further evaluate this hypothesis. Supported by NIEHS ES05651.

486 MECHANISMS DRIVING TH1- AND TH2-LIKE PRIMARY RESPONSES TO THE HAPTEN PENICILLIN

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Little is known concerning early events regulating Th1/Th2 differenciation following a single exposition to haptenic compounds. Our study evidence the role of IL-4, IL-10, IL-12, and IFN-γ in controlling primary benzylpenicilloyl (BPO)-specific IgG1 and IgG2a responses in Balb/c and SJL mice. In SJL mice, sc injection of BPO coupled to tetanus toxoid (BPO-TT) induced a high and very early expression of IL-4 mRNA, a rapid increase of IL-10 mRNA expression, and a low IL-12 p40 mRNA induction in lymph node cells. These expressions were correlated with a high anti-BPO IgG1 and no anti-BPO IgG2a production. Thus, IL-4 and IL-10 allow BPO-specific IgG1 production and inhibit anti-BPO IgG2a production, despite expression of IFN-γ mRNA. In Balb/c, a lower and delayed IL-4 and IL-10 mRNA expression and a higher IL-12 mRNA expression correlated with a high anti-BPO IgG2a response and a lower anti-BPO IgG1 response. In addition, basal IL-10 mRNA level was significantly higher in SJL than in Balb/c. *In vivo* neutralization of IL-4 and/or IL-10 in SJL mice, as well as administration of IL-4 and/or IL-10 in Balb/c mice allowed to demonstrate that both cytokines play a critical role in controlling Th1- or Th2-type primary responses to penicillin. This might have implications in controlling drug-hypersensitivity in humans.

487 ROLE OF IgG2 IN TRIMELLITIC ANHYDRIDE (TMA)-INDUCED PULMONARY HYPERSENSITIVITY IN THE GUINEA PIG

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Sponsor: K B Wallace

Actively sensitized guinea pigs respond to intratracheal challenge with TMA conjugated to guinea pig serum albumin (TMA-GPSA) with an immediate bronchoconstriction and delayed cellular infiltration into the lungs; responses characteristic of occupational asthma in humans. These antigen-induced responses have been shown to be mediated by IgG1 in the guinea pig. The purpose of this study was to determine whether TMA-specific IgG2 was important in TMA-induced pulmonary hypersensitivity. Passively sensitizing with both IgG subclasses (IgG1 plus IgG2, purified by anionic exchange chromatography) resulted in a significantly greater TMA-induced increase in pulmonary resistance (66.7 ± 11.2%) compared with animals sensitized with IgG1 alone (11.6 ± 1.9%) or IgG2 by itself (1 ± 1%). The number of eosinophils in the bronchoalveolar lavage (BAL) was also significantly greater after TMA-GPSA challenge in animals sensitized with IgG1 plus IgG2 ($5.1 \pm 1.2 \times 10^6$) compared to control animals ($1.4 \pm 0.4 \times 10^6$) or animals sensitized with only IgG1 ($2.2 \pm 0.4 \times 10^6$) or only IgG2 ($3.2 \pm 1.3 \times 10^6$). In addition, a significant increase in total protein in the BAL was noted in guinea pigs passively sensitized with IgG1 plus IgG2 (6.9 ± 0.6 mg) when compared to the other treatments (4.8 ± 0.3 and 5.2 ± 0.3 mg for IgG1 and IgG2, respectively, and 5.1 ± 0.7 mg for control). These data demonstrate an augmentation of IgG1-mediated responses by the addition of IgG2 and suggest a significant role for both IgG subclasses in TMA-induced pulmonary hypersensitivity.

488 FURTHER DEVELOPMENT AND CHARACTERIZATION OF A MOUSE INTRANASAL METHOD FOR COMPARATIVE IMMUNOGENICITY TESTING OF ENZYMES

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A mouse intranasal (i.n.) dosing method was developed to test the immunogenicity of enzymes. In this method, various doses of the enzymes were administered in a detergent matrix, via intranasal installation on days 1, 3, and 10, with serum samples collected on day 15. The sera were then analyzed for enzyme specific IgG1 antibody by an ELISA. The protease enzyme Alcalase® has been used as a prototype for development and characterization of the model. High titer (>1:1000 at some doses) IgG1 responses were detected when Alcalase was administered in either saline or a detergent based matrix. The detergent matrix enhanced the response approximately 10 fold based on ED_{50} comparison. Antigen-specific allergic antibody (i.e., IgE) was detectable in this method, but required several weeks of dosing to produce a low level response. Therefore, the IgG1 response has been used as a marker of immunogenicity. Several inbred, outbred, and hybrid mouse strains have been tested in the model. High responsiveness was seen in 2 hybrid strains (BDF1 and CB6F1) that share the C57B1/6 strain ($H\text{-}2^b$) parent. As expected, inbred C57B1/6 mice were also high responders. Balb/c ($H\text{-}2^d$) mice responded poorly. The range of potencies detected among enzymes of different classes (protease and nonprotease) has been approximately 40 fold (0.1X to 4.8X compared to Alcalase). Additional studies with nonenzyme allergenic proteins have been initiated to see if a greater range can be detected. Comparative (vs. Alcalase) potency determinations for several individual enzymes have related favorably to prior data from the guinea pig intratracheal test (GPIT). Given the simplicity and low cost of the i.n. model compared to the GPIT, these results support continued development of the method as an alternative approach for assessing the immunogenicity of proteins.

489 DIFFERENTIAL EXPRESSION OF T CELL ACTIVATION MARKERS (CD62L, CD44, CD45RB, CD25) IN DRAINING LYMPH NODES OF MICE FOLLOWING EXPOSURE TO ALLERGENS AND IRRITANTS

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The local lymph node assay (LLNA) uses cellular proliferation in draining lymph nodes (DLN) following topical exposure to chemicals for assessing contact sensitization potential. However, proliferation has been observed with some irritants, e.g., benzalkonium chloride (BC). To characterize further events in the LLNA and distinguish allergen- from irritant-induced proliferation, analysis of T cell activation markers was performed. Mice were treated on the ears for three consecutive days and phenotypic analysis of DLN cells made 48 hours following the final treatment. In mice treated with 0.5% picryl chloride (TNCB), the percent of $CD4^+$ T cells expressing $CD62L^{hi}$ (L-selectin) decreased while the percent of $CD4^+$ cells expressing $CD44^{hi}$ (H-CAM) increased. There was a 5.9-fold increase in the percentage of $CD4^+CD44^{hi}CD62L^{lo}$ cells in TNCB treated DLN compared to the vehicle control. In contrast, mice treated with the irritant, BC at 2.0%, caused neither an increase in $CD4^+CD44^{hi}$ cells or in $CD4^+CD62L^{lo}$ cells. $CD4^+$ cells from TNCB

treated mice also exhibited a 2-fold increase in the percent of CD45RB^{lo} cells compared to the vehicle control, whereas only a 1.3-fold increase was observed in BC treated mice. Mice treated with TNCB exhibited an increase in the number of large CD4$^+$ cells (forward scatter) and a slight increase in percent of CD4$^+$CD25$^+$ cells. Expression of CD44, CD45RB, CD25 and CD62L were similar in DLN cells of vehicle treated and naive mice. Additional irritants and allergens gave qualitatively similar profiles. These findings support the conduct of additional experiments to confirm that T cell activation markers are useful to better differentiate allergen and irritant responses in DLN.

490 CUTANEOUS TUMOR NECROSIS FACTOR-α (TNF-α) PRODUCTION AND LOCALIZATION OF TNF-α mRNA FOLLOWING SKIN SENSITIZATION OF MICE

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Epidermal cytokines play an essential role in cutaneous immune function and in the induction of contact sensitization. Tumor necrosis factor-α (TNF-α), an inducible product of keratinocytes, has been shown to be necessary for the migration of Langerhans cells from the skin and for the development of optimal contact sensitization. In the present study we have examined the expression of this cytokine local to the site of exposure to the contact allergen oxazolone. Production of TNF-α protein, as measured using the L929 bioassay, was increased within one hour of treatment, was maximal by two hours and declined rapidly thereafter. Patterns of mRNA expression were investigated by non-isotopic *insitu* hybridization with image analysis. In untreated skin TNF-α mRNA was expressed constitutively only in the hair follicles and sebaceous glands of the dermis. Highly localized and transient expression of epidermal TNF-α mRNA was observed as early as 10 minutes following exposure to oxazolone. These data confirm that contact allergens induce the expression of epidermal TNF-α and indicate that such expression is transient and highly regulated.

491 FURTHER EVALUATION OF THE MURINE LOCAL LYMPH NODE ASSAY WITH TEN CHEMICALS AND COMPARISON WITH GUINEA PIG AND HUMAN TEST DATA

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The murine local lymph node assay (LLNA) evaluates the sensitization potential of chemicals by assessing the cell proliferation occurring in the auricular lymph nodes following topical application of a chemical to the ears. The objective of these experiments was to evaluate the LLNA with known non-, moderate, and severe dermal sensitizers and compare data to guinea pig and human patch test results. Female CBA/J mice were exposed to vehicle or test chemical on the dorsum of both ears for 3 days; injected iv with 2 μCi/mouse 125-lododeoxyuridine 3 days later; and nodes removed after 5 hr. The following chemicals were evaluated: isopropanol, para-aminobenzoic acid (pABA), dimethyl isophthalate (DMI), 2-hydroxypropyl methacrylate (2-HPMA), 4-hydroxybenzoic acid (4-HBA), glycerol, 4-chloroaniline (4-CA), isophorone diisocyanate (IPDI), diphenylmethane-4,4'-diisocyanate (MDI), and para-phenylenediamine (p-PDA). The OECD recommended positive control, α-hexylcinnamic aldehyde, was used in all experiments. Treated/Control (T/C) values ≥3 or statistical significance ($p \leq 0.01$) indicated a positive response. With the exception of two chemicals (4-CA and 4-HB), there was good concordance with data derived from guinea-pig maximization tests (GPMT) and, when available, human patch tests. 4-HB and 4-CA, classified as a mild and moderate sensitizer, respectively, using the GPMT, were negative in the LLNA. These findings continue to demonstrate the appropriateness of the LLNA as a screen for identifying chemicals with sensitization potential.

492 INCREASE IN PULMONARY MAST CELLS OF GUINEA PIGS AFTER SENSITIZATION TO TOLUENE DIISOCYANATE

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Toluene diisocyanate (TDI) is the leading cause of occupational asthma. The toxicity of TDI, including respiratory irritation and sensitization, has been broadly studied, but the mechanism(s) remain unknown. We have developed a guinea pig model to investigate the mechanisms of the hypersensitivity response. In the exposure paradigm, animals were exposed to 1 ppm TDI 3h/day on 5 consecutive days. High titers of hypersensitivity antibodies (10^3–10^4) were evident by day 14. In the current study we examined the lungs of animals for evidence of mast cell involvement. Twenty guinea pigs were divided into four groups. The first two groups received 1 ppm TDI following the sensitization paradigm. Group 1 was sacrificed on day 5, the second group was challenged with 0.10–0.25 ppm TDI on day 23. The third group received only TDI challenge, and the fourth group received neither induction, nor elicitation exposure to TDI. Lungs were fixed in buffered formalin and embedded in paraffin. Two methods were used to quantify the changes in mast cells: toluidine blue staining, and binding of fluorescein isothiocyanate conjugated avidin. Mast cells were significantly increased in guinea pigs that were sensitized and challenged with TDI. The greatest number of mast cells was found in the bronchial region, around the small blood vessels, in the lamina propria, and in the submucosal region. A correlation was evident between TDI-specific IgG_1 antibodies and mast cell number. These results are consistent with clinical data which have indicated that individuals with TDI asthma have an increased number of mast cells as detected in tissue obtained by the bronchial biopsies. This study extends the findings of the guinea pig model and suggests the importance of mast cell activity in TDI asthma. Supported by NIEHS ES05651.

493 THE POTENTIAL USE OF LYMPHOCYTE PHENOTYPE FROM DRAINING LYMPH NODES OF XENOBIOTIC EXPOSED ANIMALS IN IDENTIFING CONTACT AND RESPIRATORY SENSITIZERS

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The purpose of this study was to describe the type and time course of the lymph node cell proliferation after sensitization with known respiratory, TDI and TMA, and contact, DNFB, sensitizers. Xenobiotics were applied topically to the ears of mice over a time course of 1 to 10 days. Doses chosen for sensitization were 50% TMA, 5% TDI and .15% DNFB. Cells from the draining lymph nodes were stained with the following markers: R-PE-CD3-E, R-PE-CD4, FITC- CD8a, FITC-IG, FITC-IgM, FITC-IgE and FITC-IgG2a. For all three xenobiotics CD4+ and CD8+ positive cell numbers began to increase on day 2 of exposure and continually rose throughout the experimental period. For the immunoglobulin surface markers, there was a gradual increase in Ig and IgM positive cells throughout the time course of the study. Class switching occurring on day 7 was indicated by elevations of IgE and IgG2a. For both respiratory sensitizers, TMA and TDI, numbers of IgE positive cells remained low until day 7 at which time there was a rapid increase. Over the experimental period, the number of IgE positive cells increased up to 46-fold for TDI and 176-fold for TMA. In DNFB exposed animals, the number of IgE positive cells showed only an 8-fold increase. An increase in IgG2a positive cells was also seen on day 7 with an approximate 7-fold increase in animals dosed with TDI, an approximate 18-fold increase in animals dosed with TMA and a 10-fold increase in IgG2a positive cells in DNFB treated animals. The ratio of IgE positive cells to IgG2a positive cells in the draining lymph node of xenobiotic exposed mice may provide an additional means for differentiating Type I and Type IV hypersensitivity responses. These studies were conducted at the Medical College of Virginia Immunotoxicology Laboratory under NIEHS Contract ES 05288.

494 EVALUATION OF THE MURINE IgE TEST FOR ASSESSING POTENTIAL RESPIRATORY SENSITIZERS USING A MULTIPLE INDUCTION REGIMEN

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There has been increasing concern over the potential of industrial chemicals to produce allergic reactions in the respiratory tract. The murine serum IgE test is under evaluation as a means to identify respiratory sensitizers (RS). In this study, 10 groups of BALB/c female mice (n = 7) were treated on Day 0 at two dose levels with 50 μl of either diphenylmethane-4, 4'-diisocyanate (MDI); toluene 2, 4'-diisocyanate (TDI); oxazolone (OX); maleic anhydride (MA); or methyl acrylate (MAc) on each shaved flank. An untreated group and an acetone/olive oil vehicle (VH) group were included. MDI, TDI and MA are known human RS and dermal sensitizers (DS). OX is a human DS; MAc is a delayed human DS. On day 3, 25 μl of each test material or VH was again applied to each shaved flank. On day 7, a 12.5 μl aliquot of test material was applied to the dorsal and ventral surface of each ear. The animals

were sacrificed on day 14 and serum was analyzed for IgE using an ELISA. At the high dose level, all test materials with the exception of MAc were significantly higher ($p<0.01$) than the VH. OX also produced a positive response. Although the results indicate a robust response for RS, a lack of specificity to distinguish DS vs RS was apparent. Further efforts which may be needed include challenge by the inhalation rather than dermal route and/or evaluation of hapten specific antibodies.

495 ASSESSMENT OF THE SENSITIZING POTENTIAL OF FORMALDEHYDE

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It is well established that formaldehyde is able to cause allergic contact dermatitis. Less certain is the potential of this chemical to induce sensitization of the respiratory tract and allergic asthma. Consistent with skin sensitizing activity formaldehyde was found in the present investigations to induce strong positive responses in the guinea pig maximization and Buehler occluded patch tests and in the local lymph node assay. The mouse IgE test is a novel method for the identification of potential chemical respiratory allergens in which activity is measured as a function of the ability of topically applied materials to stimulate an increase in the serum concentration of IgE in BALB/c mice. At concentrations that stimulated vigorous responses in the local lymph node assay, formaldehyde was inactive in the mouse IgE test. In supplementary experiments the pattern of cytokine production by draining lymph node cells induced by topical exposure of mice to formaldehyde was examined. Responses to this chemical were characterized by the production of comparatively high concentrations of the type 1 cytokine interferon-γ, but only low levels of the type 2 cytokines interleukins 4 and 10; a pattern consistent with the selective stimulation of Th1 T helper cells. These data confirm the skin sensitizing activity of formaldehyde, but indicate the absence of a significant potential to cause sensitization of the respiratory tract.

496 CHARACTERIZATION AND CLASSIFICATION OF CHEMICAL ALLERGENS AS A FUNCTION OF CYTOKINE SECRETION PROFILES

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We have demonstrated previously that repeated topical exposure of mice to chemical contact or respiratory allergens induces characteristic cytokine secretion profiles consistent with the selective activation of T helper 1 (Th1)- and Th2-type cells, respectively. Concentrations of the respiratory allergens trimellitic anhydride (TMA) and toluene diisocyanate (TDI) and the contact sensitizers oxazolone and dinitrochlorobenzene (DNCB) were used which stimulated equivalent levels of immunogenicity with respect to lymph node cell (LNC) proliferation. Under such conditions of exposure, TMA- and TDI-activated LNC expressed high concentrations of the Th2-type cytokines interleukin 10 and interleukin 4, but low levels of the Th1 cell product interferon γ. The converse pattern of cytokine production was observed for oxazolone- and DNCB-stimulated LNC. In subsequent experiments we have examined dose-response relationships for a wider range of chemical allergens. In each case, cytokine production profiles were compared with those observed in LNC derived from animals exposed concurrently to the respiratory allergen TMA, or the contact allergen DNCB. At all concentrations tested, the contact allergens eugenol and isoeugenol provoked a Th1-type cytokine secretion profile while a Th2-type pattern was induced by exposure to the respiratory allergens cyanuric chloride and diphenylmethane diisocyanate. These data suggest that it may be possible to characterize and classify different types of chemical allergen as a function of LNC cytokine production profiles induced following topical exposure.

497 ATTENUATION OF SILICA-INDUCED CYTOTOXICITY BY LAZAROID (U-75412E)

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Lazaroids (21-aminosteroids) are potent scavengers of oxygen radicals (OR) and inhibitors of lipid peroxidation. They have been shown to provide protection from OR induced injury. Crystalline silica (silica) a potent cytotoxic agent is known to cause pulmonary fibrosis in animals and man. We have shown previously that ORs are involved in silica-induced pulmonary injury and inflammation. In the present study, we investigated the effectiveness of lazaroid (U-75412E) in the amelioration of silica-induced cytotoxicity in rat alveolar macrophages (AM). To test the efficacy of lazaroid in attenuating in vitro silica-induced cytotoxicity, we measured lactate dehydrogenase, β-N-acetyl glucosaminidase, superoxide dismutase, glutathione peroxidase, and hydrogen peroxidase release from AMs as indicators of cytotoxicity in the presence and absence of lazaroid. We also studied the ability of lazaroid to scavenge hydroxyl radicals (OH) generated by silica from hydrogen peroxide. Lazaroid protected AMs from silica-induced cytotoxicity and injury. Lazaroid also exhibited a dose dependent ability to scavenge OH. Results of these in vitro studies suggest that administration of lazaroid in silica-induced lung injury may protect the lung against oxidant injury.

498 LUNG DEPOSITION AND ALVEOLAR MACROPHAGE UPTAKE OF MICROPARTICULATES FROM SUSPENSION AEROSOLS IN GUINEA PIGS

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Size dependency of uptake, disposition, residence time and location of particles is implicated in local inflammatory and chronic diseases of the lungs. Particulate aerosols that deposit in the periphery of the lungs adjacent to alveolar macrophages (AM) will be phagocytosed. Aqueous suspensions of fluorescent polystyrene beads (Dp = 1 or 3μm) were nebulized. Mass median aerodynamic diameters (MMAD) and geometric standard deviations (GSD) were determined by inertial impaction. Aerosols were administered to guinea pigs for 10 or 30 minutes. AM were harvested from bronchoalveolar lavage fluid at 0 and 6 hours. Macrophage uptake of microparticles was assessed by fluorescence microscopy. Aerosols were characterized from the nebulizer (Dp = 1μm, MMAD = 2.1, GSD = 3.4; Dp = 3μm, MMAD = 4.3, GSD = 2.2) and from the inhalation exposure chamber during delivery to the guinea pigs (Dp = 1μm, MMAD = 1.5, GSD = 2.4; Dp = 3μm, MMAD = 2.9, GSD = 1.9). The difference between nebulizer and exposure chamber output particle size may be explained by the loss of large droplets in transit through the chamber. These data define the upper and lower limits of aerosol size to which the animals were exposed. Phagocytosis of 1μm particles by AM was evident 6 hours after aerosol exposure. Phagocytosis of 3μm microparticles could not be detected 6 hours after a ten minute exposure to nebulized aerosol, but thirty minutes of aerosol delivery resulted in detectable phagocytosis 6 hours after exposure. These studies demonstrate a size dependency of uptake of microparticles delivered as aerosols to the lungs. Future studies will evaluate the dose and kinetics of disposition of aerosol particles.

499 ACUTE LUNG RESPONSES TO URBAN DUST AND OZONE IN FISCHER 344 RATS

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We have studied in a short-term animal assay the pulmonary effects of ambient air particles in interaction with ozone. Fischer 344 male rats (n = 5–12) were exposed for 4th by nose-only route to clean air, ozone (0.8 ppm O_3), Ottawa urban dust EHC93 (50 mg/m^3; MMAD = 1.4 μm, GSD = 1.9), or O_3 plus urban dust on a single day or 3 consecutive days. Bronchoalveolar lavage (BAL) was performed after 24h recovery in air. Exposure to O_3 resulted in elevated protein, fibronectin (FN) and alkaline phosphatase (AKP) in BAL fluid, indicating lung injury. EHC93 alone did not increase BAL protein, FN or AKP, nor did it exacerbate these effects of O_3 following co-exposure. However, the BAL neutrophilic infiltration induced by O_3 was increased by EHC93 in the 1-day exposure group. EHC93 alone did not increase BAL neutrophils. BAL macrophages (MAC) were reduced after 3-day exposure to EHC93. MAC viability was not affected by any of the *in vivo* treatments. O_3 inhalation decreased phagocytic activity and TNF-α production by MAC. EHC93 alone decreased TNF-α secretion and the LPS-stimulated nitric oxide production by MAC. While both O_3 and EHC93 separately could alter some aspects of MAC physiology, these cellular effects appeared to be attenuated by co-exposure of the rats to O_3 plus EHC93. There were no clear cumulative effects of 3-day exposure *vs* 1-day exposure. The data indicate that although inhaled urban dust has a low acute pulmonary toxicity, it can depress some key functions of MAC. Toxicological interaction with O_3 appears complex and warrants further investigation. Supported by Health Canada (K281235).

500 EFFECTS OF α-KETOGLUTARIC ACID ON THE DISTRIBUTION OF CYANIDE AND ACIDOSIS ASSOCIATED WITH CYANIDE INTOXICATION

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Alpha-ketoglutaric acid is a potent antagonist of cyanide poisoning. The antidotal effectiveness of this agent may be based, in part, upon the ability of α-ketoglutaric acid to bind cyanide nucleophiles in the vascular system, thus decreasing cyanide (CN) distribution to the vital tissues. Such decreased distribution would result in a diminution of the consequential histotoxic hypoxia caused by CN intoxication. To test this hypothesis, groups of male ICR mice (24–28 g) were injected i.p. with 2 g/kg of α-ketoglutaric acid, or physiological saline solution (10 ml/kg). Ten minutes later, the same mice were challenged with 5.0 mg/kg KCN, i.p., having a specific activity of 0.2 μCi/kg (C-14 radiolabeled KCN). Tissues were collected at select time intervals after KCN challenge. CN concentrations in the plasma were substantially higher ($p<.05$) in α-ketoglutaric acid pretreated mice than in the saline pretreated controls. The distribution of CN to the brainstem and heart was significantly reduced by pretreatment with α-ketoglutaric acid. Histotoxic hypoxia, as gauged by the degree of metabolic acidosis, was significantly reduced by α-ketoglutaric acid. Alpha-ketoglutaric acid was found to afford significantly more protection against CN induced deaths than therapeutic doses of cobalt edetate or sodium pyruvate, which also bind CN.

501 SUBSTANCE P ANTAGONIST, CP-96,345, POTENTIATES JP-8 JET FUEL INDUCED LUNG INJURY C57BL6 MICE

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In a simulated military flightline exposure protocol, C57BL6 mice were used to investigate the pulmonary effects of JP-8 jet fuel inhalation. Male C57BL6 mice were exposed nose-only daily to an average JP-8 concentration of 1092 mg/m^3 (BHD, n=8) or ambient air (BLC, n=8) for seven days. Alveolar epithelial permeability, bronchoalveolar lavage total protein, and lung compliance were measured as indices of pulmonary toxicity. Alveolar epithelial permeability was measured by the clearance of instilled technetium labeled diethylenetriamine pentaacetate (^{99m}Tc-DTPA). BHD (0.357 ± 0.12 %/min) and BLC (0.344 ± 0.07 %/min) exposure groups had similar alveolar epithelial permeability. In support of the ^{99m}Tc-DTPA results, BHD (34.38 ± 4.5 μg/ml) and BLC (43.85 ± 4.4 μg/ml) exposure groups had statistically similar bronchoalveolar lavage (BAL) total protein levels. BHD (3.94 ± 0.10 ml/cmH_2O/kg) and BLC (3.57 ± 0.11 ml/cmH_2O/kg) mice also had similar lung compliance. These results suggest that JP-8 jet fuel inhalation is non-toxic at this dose. Male C57BL6 mice were also pretreated with 2.5 mg/kg i.p. or the selective substance P antagonist CP-96,345 15 minutes prior to each JP-8 jet fuel (BCP, n=12, 1269 mg/m^3/day) or ambient air (LCP, n=6) exposure for seven days. BCP mice had significant increases in ^{99m}Tc-DTPA clearance (1.254 ± 0.18 %/min) and in BAL total protein levels (62.48 ± 8.3 μg/ml) as compared to BLC, BHD, and LCP (0.502 ± 0.34 %/min, 27.17 ± 3.10 μg/ml) exposure groups. The BCP mice had a significant reduction in lung compliance (3.15 ± 0.10 ml/cmH_2O/kg) as compared to BLC and BHD exposure groups, but not LCP (3.45 ± 0.34 ml/cmH_2O/kg). The reduced comliance could be attributed to CP-96,345 drug. Light microscopy revealed signs of edema, airway inflammation, and red blood cell infiltration in the BCP mice, while not observed in the other groups. The results suggest that the substance P receptor has an important role in preventing JP-8 jet fuel induced pulmonary toxicity. Supported by USAFOSR F49620-94-1-0297 and AASERT.

502 THE INFLUENCE OF TRACHEOBRONCHIAL REGION VOLUME ON REACTIVE GAS UPTAKE AND INTERSPECIES DOSE EXTRAPOLATIONS

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With the increasing use of dosimetry models for quantitative dose extrapolations, it is important that model parameters are based on reliable anatomical data. In this study, the influence of tracheobronchial (TB) volume on fractional uptake, proximal alveolar region (PAR) dose, and the ratio of rat to human PAR doses (dose ratio) was investigated using a dosimetry model to simulate the uptake of reactive gases in the lower respiratory tracts of rats and humans. The PAR was chosen because it is the site of the most severe lesions in laboratory animals exposed to O_3 and NO_2. PAR dose ratios can be used to establish exposure concentrations that yield the same PAR dose in different species, thereby facilitating extrapolation of animal toxicological results to humans. The influence of TB volume was explored using different ventilatory parameters, TB region mass transfer coefficients, and whether or not the TB region expanded and contracted. Results for TB volumes that were two standard deviations (2SD) less than or greater than mean functional residual capacity volume (FRC) were compared to predictions for the mean. Doses were predicted to be up to 57% smaller (larger FRC) and up to 197% larger (smaller FRC) than doses associated with the mean TB volume. For PAR dose ratios, the ± 2DS range about the mean TB region volume can lead to ratios which vary by up to a factor of 20, indicating that PAR dose ratios are very sensitive to TB region volume. This study indicate that a better characterization of the role of TB region volume is important for an improved understanding of respiratory tract dosimetry of reactive gases and for decreasing uncertainties in inter- and intraspecies extrapolations of dose associated with toxicological effects. (This abstract does not necessarily reflect EPA policy.)

503 FORMATION OF TOBACCO SMOKE-INDUCED HEMOGLOBIN ADDUCTS IN RATS

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Hemoglobin adducts have been used as indicators of cumulative exposure to chemicals. Cigarette smoke, which is a complex mixture of chemicals, can potentially generate electrophiles *in vivo* and interact with hemoglobin (Hb) to form a variety of adducts. The present study examined the formation of three Hb adducts in a rat model following experimental exposure to tobacco smoke. Sprague Dawley rats were exposed to sidestream cigarette smoke (SS-CS) for 6 hrs for 1 day or 6 hrs each day for 1 and 3 months in a whole body exposure chamber set at a concentration of 9.3 ± 1 mg TPM /m^3. Sixteen hrs after the last exposure, the animals were sacrificed and RBC isolated for analysis of Hb adducts. Three adducts, one formed by interaction of acrylonitrile with valine residues (AN-Val) and two formed by interaction of 4-aminobiphenyl (ABP-Cys) or benzo(a)pyrene (BP-Cys) with cysteine residues, were identified and measured by GC/MS and HPLC. Hb adducts could be detected even after 1 day of exposure to SS-CS. Daily smoke exposure for 1 month increased the AN-Val, ABP-Cys and BP-tetrol-Cys adducts in exposed animals by about 2, 40 and 8-fold, respectively, in comparison to control levels of 17.3 ± 0.02 pg AN-Val, 3.82 ± 0.91 pg ABP-Cys and 5.09 ± 1.2 pmoles BP-tetrol-Cys /g Hb. These results support the use of Hb adducts as potential biomarkers of tobacco smoke exposure (KTRB 5-41110, and EPA-3D2320NATA).

504 ENHANCEMENT OF AIRWAY EPITHELIAL Na^+, K^+-ATPase ACTIVITY IN RESPONSE TO NITROGEN DIOXIDE EXPOSURE INVOLVES INHIBITION OF PROTEIN PHOSPHATASE 1

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Acute exposure to elevated levels of nitrogen dioxide (NO_2) has been linked to airway epithelial injury and development of airway hyperreactivity. In vitro exposure of airway epithelium to NO_2 has recently been shown to enhance Na^+, K^+ -ATPase (sodium pump) activity. Pump activity is thought to be regulated by reversible phosphorylation of the catalytic α subunit. Oxidant stress, such as that imposed by NO_2, may potentiate phosphorylation through inhibition of protein phosphatase (PP) activity. We have examined the role of protein phosphatases in the NO_2-induced enhancement of airway epithelial sodium pump activity. Guinea pig tracheobronchial epithelial (GPTE) cells were cultured in an air-interface to form tight monolayers that actively transport ions transepithelially. GPTE monolayers were incubated with PP inhibitors, 10 nM calyculin A (an equipotent inhbitor of PP1 and PP2A) or 5 nM okadaic acid (a selective inhibitor of PP2A), during a 1 hr exposure to air or 5 ppm NO_2. Ouabain-sensitive ^{86}Rb uptake was measured as an index of sodium pump activity. NO_2 exposure enhanced ^{86}Rb uptake to 319.0 ± 7.3 nmoles ^{86}Rb/mg protein/30 min in comparison with air-control value of 221.6 ± 26.28. ^{86}Rb uptake by air-control monolayers treated with calyculin A was enhanced to 324.2 ± 5.2, while no change was found with monolayers treated with okadaic acid. ^{86}Rb uptake by NO_2-exposed monolayers treated with calyculin A or okadaic acid were unchanged from NO_2 alone. Enhanced ^{86}Rb uptake seen in calyculin A-treated air-control

monolayers may be due to an inhibition of PP1, as okadaic acid had no effect, suggesting that PP2A is not involved. If NO_2 exposure inhibited PP1 activity, calyculin A would have no effect. Enhanced sodium pump activity found with NO_2 exposure is thus likely to reflect inhibition of PP1. Supported by NIH Grants 46943 and 38658.

505 CYTOKINE ALTERATIONS ASSOCIATED WITH AMIODARONE-INDUCED PULMONARY TOXICITY IN RATS

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Amiodarone (AD) is an antiarrthymic drug with numerous side effects, the most serious being the development of pulmonary toxicity. We have previously shown that a single intratracheal (IT) instillation of AD to Fischer 344 rats results in pulmonary fibrosis within 6 weeks of treatment. Presently, the mechanism(s) of AD-induced pulmonary toxicity is unknown. It is thought that cytokines may play a role in its toxicity. To investigate this possibility, female Fischer 344 rats were given a single IT instillation of AD (6.25mg/kg), or its metabolite desethylamiodarone (5mg/kg) or vehicle (sterile water). At 1,2,3 and 6 weeks following treatment the lungs were lavaged and the recovered bronchoalveolar (BAL) cells were counted and identified. The alveolar macrophages were isolated as monolayer and cultured for 24 hrs. The spent media were collected for the analyses of TNF-α and TGF-β content using bioassays. Total and differential BAL cell counts showed that AD treatment induced a lymphocytic alveolitis in rats. In contrast, desethylamiodarone-treated rats developed a neutrophilic alveolitis. The secreted levels of TNF-α and TGF-β were unchanged in macrophage cultures from desethylamiodarone-treated rats. However, a significant difference in the levels of TNF-α in macrophage cultures was observed between control and AD-treated groups. Further, the levels of TGF-β, while not statistically different from controls, tended to be higher in cultures from AD-treated rats. These results suggest that TNF-α and TGF-β may have a role in the AD-induced pulmonary toxicity.

506 STRAIN DIFFERENCES IN SUSCEPTIBILITY OF MICE TO STYRENE-INDUCED HEPATOTOXICITY AND PNEUMOTOXICITY

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It has previously been shown that the responsiveness of mice to styrene-induced hepatotoxicity varies with strain. It has also been demonstrated that styrene can be pneumotoxic in non-Swiss albino (NSA) mice. The purpose of these studies was to compare strains of mice for these two endpoints. Adult male NSA, A/J, C57BL/6 and CD-1 (Swiss) mice were administered styrene (800 mg/kg) or styrene oxide (300 mg/kg) ip. After 24 hr, hepatotoxicity was assessed by measuring serum sorbitol dehydrogenase (SDH) and pneumotoxicity by determining γ-glutamyl transpeptidase (GGT) and lactate dehydrogenase (LDH) activities in bronchoalveolar lavage fluid. A/J and C57BL/6 mice were similar to NSA mice in response. CD-1 mice were much less susceptible than were NSA mice to both the hepatotoxicity and pneumotoxicity of styrene. Pyridine (200 mg/kg, ip, 18 hr before styrene), which was an effective inducer of hepatic CYP2E1 in both strains, increased the hepatotoxicity of styrene, especially in the CD-1 mice, but it had a greater effect on the pulmonary toxicity in NSA mice. These studies confirm the strain differences in susceptibility to liver damage and extend these to the lung. (Supported in part by NIH grant ES04362 and the Styrene Information and Research Center.)

507 N-ACETYLCYSTEINE AEROSOL THERAPY FOR RESPIRATORY FIBROSIS FROM BLEOMYCIN

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Exposure to respiratory toxicants causes fibrotic lung disease. Treatment of existing fibrosis with amino acid analogs that disrupt collagen or prevent parenchymal cell oxidation are therapeutic. We studied if compounds delivered to the respiratory tract as propellant-driven (P-D) aerosols are therapeutic for induced pulmonary fibrosis. Mice exposed to a fibrogenic dose of 0.1 unit of bleomycin by intratracheal instillation were then treated with 4 P-D aerosol exposures of N-acetylcysteine in a nose-only chamber 7–21 days after bleomycin (Brown and Pickrell, J Immunol Meth 176: 203– 212, 1994). The aerosol was >60% respirable (aerodynamic diameter ≤5 uM) as indicated by Andersen Impactor. The N-acetylcysteine treatments ameliorated >55% of the lung weight increases and >45% of the lung collagen increases associated with bleomycin-only exposure. Four of 5 mice given bleomycin alone had macroscopic lung lesions, compared to only 1 of 5 mice given N-acetylcysteine therapy. P-D N-acetylcysteine aerosols may deter or provide therapy for individuals experiencing chemically-induced lung fibrosis. [We acknowledge research support from the State of Kansas.]

508 SIMULATION OF INSPIRATORY AIRFLOW IN THE OLFACTORY REGION OF THE F344 RAT NASAL PASSAGES

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A computational fluid dynamics (CFD) model of airflow and inhaled gas uptake has been used to predict dose to airway walls in the anterior F344 rat nasal passages (Kimbell, J.S. et al., *Toxicol. Appl. Pharmacol.* 121:253–263, 1993). However, many chemicals induce changes in the olfactory (posterior) region of the rat nose. The olfactory region was added to the anterior nose model by photographing serial-step, histological sections through the entire nasal passages of a F344 rat, digitizing airway outlines, and constructing a three-dimensional, finite element mesh of the right side of the nasal passages from nostrils to nasopharynx using inhouse software and FIDAP (Fluid Dynamics International, Evanston, IL). Anterior nasal airflow patterns predicted with the expanded model are not significantly different from airflow patterns predicted for the anterior nose alone. Major routes of airflow simulated in the posterior nose were consistent with experimental visualizations (Morgan, K.T. et al., *Toxicol. Appl. Pharmacol.* 110:225–240, 1991) and were located ventrally and adjacent to the dorsal septum and medial aspects of the first, third, and fifth ethmoturbinates (terminology from Mery, S. et al., *Toxicol. Pathol.* 22:353–372, 1994), with slow airflow occurring in posterior lateral airways. These results indicate that airflow patterns are important for transport of inhaled gases through medial posterior airways and that other factors, such as air-phase diffusion, may play a more significant role in transport to lateral regions. This model will be used to evaluate the role of airflow patterns in determining the distribution of xenobiotic-induced olfactory mucosal lesions. This information, combined with models of disposition in the airway lining, will provide comprehensive dosimetry models for extrapolating animal response data to people.

509 THE EFFICACY OF IBUPROFEN AND PENTOXIFYLLINE IN THE TREATMENT OF PHOSGENE-INDUCED ACUTE LUNG INJURY

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Phosgene, a highly reactive former warfare gas, is a deep lung irritant which produces Adult Respiratory Distress Syndrome (ARDS) - like symptoms following inhalation. Death caused by phosgene involves a latent, 6–24 hr, fulminating non-cardiogenic pulmonary edema. The following dose-ranging study was designed to determine the efficacy of a non-steroidal anti-inflammatory drug, ibuprofen (IBU), and a methylxanthine, pentoxifylline (PTX). These drugs were tested singly and in combination to treat phosgene-induced acute lung injury in rats. IBU, in concentrations of 15–300 mg/kg (i.p.), was administered to rats 30 minutes before and 1 hour after start of whole-body exposure to phosgene (80 mg/m^3 for 20 minutes). PTX, 10–120 mg/kg (i.p.), was first administered 15 minutes prior to phosgene exposure and twice more at 45 and 105 minutes after start of exposure. Five hours after phosgene inhalation, rats were euthanized, the lungs were removed and wet weight values were determined gravimetrically. IBU administered alone significantly decreased lung wet weight to body weight ratios compared with controls ($p \leq .01$) whereas PTX, at all doses tested alone, did not. In addition, the decrease in lung wet weight to body weight ratio observed with IBU + PTX could be attributed entirely to the dose of IBU employed. This is the first study to show that pre- and posttreatment with IBU can significantly reduce lung edema in rats exposed to phosgene.

510 INHALATION OF *m*-XYLENE AT THRESHOLD LIMIT VALUE (TLV) LEVELS RESULTS IN ALTERATIONS OF CYTOCHROME P450 ISOZYMES IN THE RESPIRATORY TRACT OF THE RAT

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m-Xylene inhalations (300 ppm; 6hr) have demonstrated selective inhibition of respiratory tract cytochrome P450 isozymes while resulting in minor increases in the activity of hepatic isozymes. Nasal mucosa isozymes (CYPs 2B1, 1A1, 1A2, 4B1, and 2E1) were all inhibited following exposure to 300 ppm *m*-xylene. CYPs 2B1, 1A2, 4B1, and 2E1 were also inhibited in lung. The present study examined the effects of the inhalation of a lower concentration of *m*-xylene (100 ppm; 6hr; OSHA's TLV). Following exposure to 100 ppm *m*-xylene, nasal mucosa CYP 2B1 activity was significantly decreased to values observed in animals exposed to 300 ppm. Lung CYP 2B1 activity was also significantly decreased, but the inhibition was not as great as that observed in the 300 ppm group (32% vs. 67%). CYP 1A1 activity, like 2B1 was decreased to values similar to those previously observed in the nasal mucosa with no alterations in lung or liver. The third isozyme studied, CYP 1A2, was decreased by 62% in mucosa. This decrease was less than that observed in the 300 ppm group (75% decrease). Pulmonary and hepatic CYP 1A2 activities were not altered in the TLV group. CYP 4B1 was significantly decreased in the nasal mucosa and lung in the TLV group. The decrease in pulmonary CYP 4B1 activity was equal to that observed in the 300 ppm exposure group. CYP 2E1 was not significantly decreased in nasal mucosa or lung as had previously been observed in the 300 ppm group. An increase in hepatic CYP 2E1 activity that had been observed in the 300 ppm group was not detected following exposure to the TLV. This work demonstrates that, (1) even at low levels of *m*-xylene the nasal mucosa isozymes remain sensitive to the inhibitory effects of the solvent, (2) CYPs 2B1, 1A2 and 2E1 (primarily in the lung) exhibit a dose response relationship with regards to the level of inhibition observed and (3) the inhibition of these isozymes, especially in the nasal mucosa, could result in altered xenobiotic metabolism.

511 ELEVATED SUSCEPTIBILITY TO CLARA CELL CYTOTOXICANTS DURING POSTNATAL LUNG DEVELOPMENT: A COMPARISON OF NAPHTHALENE INJURY IN THE MOUSE AND 4-IPOMEANOL INJURY IN THE RABBIT

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For most metabolically activated toxicants targeting the lung, Clara cells (CC) are the primary cell type injured by acute exposure. In adults, the degree of CC susceptibility and distribution of injury is related to the level of metabolic activity at specific sites within the lung. Despite low levels of cytochrome P450 monooxygenase activity and CC immaturity in postnatal animals, we have observed that CCs in postnatal rabbits are susceptible to 4-ipomeanol (IPO) toxicity at doses which produce no effect in adults. To test whether the difference in susceptibility is true for other species and other compounds, postnatal mice were treated with a single dose of naphthalene (NA) and acute injury compared by histopathology to postnatal rabbits treated with IPO and adults receiving similar doses. In postnatal mice, 25mg/kgNA produces extensive CC toxicity in distal bronchioles, while 50 and 100 mg/kgNA produce injury in proximal and distal airways. In adult mice, 50 and 100mg/kgNA produce minimal CC toxicity restricted to distal bronchioles. In postnatal rabbits, 1, 5 and 10 mg/kgIPO produce extensive CC toxicity in distal bronchioles in a dose-related fashion; these doses produce virtually no detectable effect in adult rabbits. We conclude that elevated postnatal susceptibility to acute Clara cell cytotoxicity by bioactivated lung toxicants is not restricted to a single species or single class of compounds. Supported by ES06700, ES04311 and ES05707.

512 DOSE RELATED AIRWAY-SPECIFIC EPITHELIAL TOXICITY OF 1-NITRONAPHTHALENE IN RATS

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Clara cells of terminal bronchioles are one of the principal targets of bioactivated pulmonary toxicants. However, we have recently observed cytotoxicity in proximal tracheobronchial airways with high doses of naphthalene in mice. To determine whether distal to proximal differences in susceptibility to pulmonary toxicants apply to other species with other compounds, male Sprague-Dawley rats were injected i.p. with 25, 50, 100 or 150 mg/kg 1-Nitronaphthalene (1-N,N) and cytotoxicity characterized by high resolution histopathology. At 25 mg/kg, only Clara cells in distal bronchioles were swollen and necrotic, and in the trachea there were small numbers of vacuolated nonciliated and ciliated cells. At 50 mg/kg there was a complete loss of Clara cells in both distal and proximal airways. Marked damage to ciliated cells was observed in proximal, but not distal airways at doses of 100 and 150 mg/kg. Basal lamina denudation of the trachea and lobar bronchi was common at the higher doses. We conclude for 1-N,N cytotoxicity in the lung that: 1) Clara cells are not the only target and that ciliated cells are also susceptible to injury; 2) the response is airway specific; 3) the response is dose-related; 4) and at higher doses tracheal epithelium is more susceptible than bronchiolar epithelium. This study was supported by NIEHS ES00628.

513 BRONCHIOLAR WOUND REPAIR FAILS IN POST-NATAL RABBITS FOLLOWING 4-IPOMEANOL-INDUCED CLARA CELL INJURY

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In adult animals, the acute bronchiolar injury produced by Clara cell-targeted xenobiotics is dose-dependent and repairs within 7–14 days. Recent studies have found elevated susceptibility to these compounds in immature Clara cells of postnatal animals. This study was done to determine if the heightened injury alters the normal course of postnatal bronchiolar maturation. Rabbits, ages 3 to 21 days old, were given a single i.p. injection of 4-ipomeanol (5mg/kg) and were killed at 4 or 6 weeks of age. At both time points, the abundance of terminal bronchiolar (TB) cuboidal epithelium was decreased considerably, and many TBs were lined by squamous epithelium. Of the animals killed at 6 weeks, almost 100% of the TBs were lined by squamous epithelium in the 7-day-treated group, approximately 40% in the 3-day-treated, and 0% in the controls. Cellular expression of Clara cell differentiation markers (Clara cell secretory protein and cytochrome P450 monooxygenases) in the treated animals was decreased also. TBs were identified by architectural organization and components of the extracellular matrix. In the distal two generations of bronchioles, smooth muscle was rare, and the matrix was composed of collagen I, IV and little elastin. We conclude that injury to Clara cells during differentiation may produce a long-lasting impediment to the normal course of postnatal bronchiolar maturation. Supported by ES06700 and ES043211.

514 DOES POSTNATAL PULMONARY DEVELOPMENT OF XENOBIOTIC METABOLIZING ENZYMES INCREASE SUSCEPTIBILITY TO BIOACTIVATED CLARA CELL CYTOTOXICANTS?

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The lung is a major target for environmental contaminants bioactivated by P450 (CYP) monooxygenases. Current standards for these contaminants are based on risk assessments from adult data. There is little information available on the relative susceptibility of children to these compounds. We have previously found that young mice (7- and 14-day old) are more susceptible to injury by the bioactivated Clara cell toxicant naphthalene (NA) than adult mice. This study was designed to determine if levels of xenobiotic metabolizing enzymes in immature Clara cells account for the increased sensitivity to injury. Intracellular protein expression of pulmonary CYP is lowest in 7-day old mice and highest in adult mice. Pulmonary glutathione *S*-transferase (GST) proteins have the lowest expression in 14-day old mice. Epoxide hydrolase protein is expressed at highest levels in 14-day old lungs. CYP2F activity in microdissected distal airways from 14-day old mice is only 40% of that found in airways from adult mice. GST activity is 15% and 50% of adult levels in airways from 7- and 14-day old mice, respectively. We conclude that heightened susceptibility of immature Clara cells to NA injury is not due to blatant differences in enzyme activity, but could be due to a mismatch of activating and detoxifying enzymes. The predictability of acute lung injury from bioactivated compounds in adult mice does not apply to mice during postnatal development. Supported by NIH ES06700 and ES04311.

515 EFFECTS OF SITE-SPECIFIC PARAMETERS ON RISK ESTIMATES DUE TO VAPOR MIGRATION THROUGH THE VADOSE ZONE

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To predict airborne chemical concentrations and associated risks due to the presence of soluble gasoline in free product on an existing aquifer, vapor fluxes of benzene, toluene, ethylbenzene, and xylenes through the vadose zone were modeled. The fluxes were then used as input into other models to predict both indoor and outdoor air concentrations. For the estimated flux rates to be representative of actual site conditions, physical/chemical and the biological parameters that control migration of vapors in soils were determined. The modeling of vapor migration in soil was performed using three approaches: (1) modeling vapor migration of chemicals upwards assuming no biodegradation or moisture content present, (2) modeling vapor migration upwards while accounting for moisture content in soil, and (3) modeling vapor migration upwards while accounting for both biodegradation and moisture content in soil. The resulting vapor fluxes were incorporated into the ISCST2 air dispersion model to calculate the outdoor chemical concentrations. Indoor air concentrations were calculated using a box model with the vapor fluxes as input. Exposure scenarios evaluated included an off-site residential child and adult and an off-site worker. The vapor fluxes and associated risks decreased as more site specific data were incorporated into the model. Field measurements of vapor fluxes indicated that the actual rates were most closely approximated by the case which included both actual soil moisture and biodegradation rates.

516 POLYGLUCOSE CONTAINING DIALYSIS SOLUTION -1 MONTH INTRAPERITONEAL TOXICITY STUDY IN BEAGLE DOGS

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The objective of this investigation was to assess the peritoneal toxicity of Polyglucose containing Dialysate Solution and compare the possible side effects with those of Dianeal PD4 with 3.86% Dextrose. Thirty-two Beagle dogs were assigned to 4 treatment groups and received either Dianeal PD4 with 3.86% Dextrose (reference solution), PD4 Electrolytes (vehicle solution), 7.5% Polyglucose in PD4 Electrolyte or 4% Polyglucose + 1% Amino Acids in PD4 Electrolytes. Each solution was given twice daily by peritoneal administration *via* a Lifecath® peritoneal implant at a dose volume of 40 $ml.kg^{-1}$ for 30 days. Peritoneal fluid was withdrawn from the abdominal cavity by "gravity flow" prior to each subsequent administration. Each dog was monitored for general health status, body weight profile, food and water consumption, ophthalmoscopy, electrocardiography, haematology, clinical chemistry and urinalysis. The data obtained indicated that there were no adverse clinical, haematological, biochemical or pathological differences between the groups receiving Polyglucose containing dialysis solutions and the vehicle and reference solutions. The Beagle dog is considered to be a viable model for the safety assessment of peritoneal dialysis solutions.

517 THE TOXICITY AND TOXICOKINETIC PROFILE OF LG 1-05 FOLLOWING INTRAVENOUS INFUSION IN THE CYNOMOLGUS MONKEY

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LG 1-05 (acetyl starch) is a derivative of natural starch with a molecular weight of approximately 200,000 daltons and a molar substitution of 0.5. LG 1-05 is intended for use as either a plasma expander or haemodilution agent. LG 1-05 was given *via* daily intravenous infusion (4 $h.day^{-1}$) to 36 laboratory-bred cynomolgus monkeys (*Macaca fascicularis*) at dose levels of 1.2, 2.4 and 4.8 $mg.kg^{-1}.day^{-1}$ for 28 days after which 12 animals were retained for either a 4 or 8 week post-treatment observation period. There were no clinical reactions to treatment. Biochemical data showed a marked and dose-related increase in AST; in the absence of any histological correlates, this finding is equivocal. Histology revealed foamy macrophage accumulations in several organs and tubular vacuolation/hydropic degeneration in the kidneys of animals that received 2.4 and 4.8 $mg.kg^{-1}.day^{-1}$. These changes, however, were shown to be partially reversible at the end of the recovery period. LG 1-05 was estimated in monkey serum using an Anthrone condensation method and results were expressed in mmol of glucose (breakdown product of LG 1-05). Toxicokinetic data showed peak serum levels of glucose were attained 1 h after the end of the 4 h infusion, after which the serum glucose levels showed a slow decline.

518 A COMPARATIVE STUDY OF TWO FORMS OF 5% GLUCOSE SOLUTION ADMINISTERED BY CONTINUOUS INTRAVENOUS INFUSION IN RATS FOR UP TO 56 DAYS

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Our laboratory routinely conducts continuous intravenous infusion studies in various laboratory species using indwelling catheters. Various vehicles have been demonstrated to cause venous irritancy, particularly over long durations. This study compared the potential local venous irritant effects of two formulations of 5% Glucose administered by continuous intravenous infusion for up to 56 days. Ten rats received preformulated 5% Dextrose Injection, U.S.P. and 10 other rats received a 5% formulation of D-Glucose, Anhydrous prepared aseptically in Sterile Water Injection, U.S.P., each at a rate of 2 mL/kg/h. There were no treatment-related effects on body weight, clinical condition or clinical pathology parameters. Histopathological examination revealed infusion site findings typically seen in studies employing indwelling catheters. The incidence and/or severity of the findings were greater in both groups than normally seen with infusion of saline, with the greatest incidence and/or severity in the group which received 5% D-Glucose, Anhydrous. The results suggested that 5% solutions of glucose, particularly D-Glucose, have a greater potential for venous irritancy than saline.

519 A NINETY DAY ORAL TOXICITY STUDY OF S-KETOPROFEN IN SPRAGUE-DAWLEY RATS

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Ketoprofen is a nonsteroidal anti-inflammatory drug with analgesic and antipyretic properties. In anti-inflammatory models, Ketoprofen has been shown to have inhibitory effects on prostaglandin and leukotriene synthesis, to have antibradykinin activity, as well as to have a lysosomal membrane stabilizing action. Ketoprofen is currently available as the racemate (RS), but only the S enantiomer possesses cyclooxygenase inhibitory activity. Both enantiomers (R & S) have similar concentration vs. time curves and do not appear to interact with each other. In order to assess the subchronic toxicity of the active S enantiomer, S-Ketoprofen was orally administered to one group of 40 (20/sex/group) and three groups of 60 (30/sex/group) Sprague-Dawley rats at dose levels of 0.25, 2.5, 9.0 and 0 mg/kg/day for 90 consecutive days. For comparison, (R,S)-Ketoprofen (racemate) was administered according to a similar regimen to two groups of 60 rats (30/sex/group). In addition, 10 animals/sex/group for control, mid S, high S, mid RS and high RS were maintained untreated (after day 90) for an additional 28 days as recovery groups. Clinical, hematological and clinical chemical signs of toxicity were observed in the mid and high dose groups for both S and RS. Significant gross and microscopic pathological alterations were observed in the gastrointestinal tract (stomach and small intestine, primarily the jejunum) in animals from the 2.5 and 9.0 mg/kg/day dose groups for both S and RS. Based upon the results of this work, a NOEL for S-Ketoprofen of 0.25 mg/kg/day was recommended.

520 ADVERSE EFFECTS OF TWEEN 80 IN DOGS

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Tween 80 is used as an emulsifying agent in several pharmaceutical preparations. However, intravenous and intramuscular administration of this material to laboratory dogs (Beagles) causes adverse reactions consistent with an allergic response. Intramuscular administration of one mL/kg of a preparation containing 4% Tween 80 to 32 Beagle dogs (either vehicle alone or vehicle with drug) produced facial swelling, generalized hyperemia, polydipsia and vocalization in several animals. Responses were most severe in animals receiving vehicle alone and one animal was sacrificed in extremis after exhibiting emesis, recumbency and hemorrhage from the rectum. In a second study designed to clarify the cause of these responses, three dogs received 0.5 mL/kg of a 4% Tween 80 preparation on three occasions. Responses were similar to those seen in the previous study. In a third study similar adverse signs were seen with intravenous administration of a vehicle containing 1%

Tween 80 for use in intravenous infusion. Three of the twelve dogs receiving this vehicle, with or without drug, were sacrificed as a result of Tween 80-related toxicity. Signs in these and other animals included swollen limbs, hyperemia, scleral injection, bright red mucous membranes, emesis and recumbency. Macroscopic and microscopic postmortem examinations revealed changes in the liver, kidneys, lung and skin which were attributed to Tween 80 administration. Based on these findings, it is concluded that the Beagle dog is not an appropriate animal model for toxicity evaluations of materials formulated using Tween 80.

521 TOXICOLOGIC EVALUATION OF THE PROTON PUMP INHIBITOR LY307640 IN RATS TREATED ORALLY FOR 3 MONTHS

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Sprague-Dawley (SD/Tac) rats (10/sex/dose) tolerated daily doses of LY307640 sodium (LY) equal to 25, 60, 130, or 300 mg/kg; growth and food consumption were unaffected. Plasma AUC for LY and its major thioether metabolite increased with dose. The primary target organ was the kidney, although treatment also produced histologic changes in the thyroid and stomach. The incidence and severity of multifocal basophilic cortical tubular regeneration were dose related in males (all doses) and females (≥ 60 mg/kg) and accompanied by slight-to-moderate increases in kidney weight. Minor increases in serum creatinine indicated no significant reduction in renal glomerular function. At higher doses (≥ 130 mg/kg), thyroid weight increases and/or slight thyroid follicular epithelial hypertrophy occurred and may have been due to increased metabolic clearance of thyroid hormone secondary to the observed induction of hepatic thyroxine glucuronosyl transferase. Gastric alterations, viz., expected consequences of the observed hypergastrinemia induced by the action of this potent antisecretory agent, were reflected by dose-related increases in stomach weights, as well as histologic and morphometric findings in the glandular stomach which included increased mucosal volume, and hypertrophy and hyperplasia of chromogranin A-positive enterochromaffin-like (ECL) cells in the gastric mucosa.

522 STEROL-INDUCED HEPATIC MICROGRANULOMAS

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LY295427 is a sterol compound being developed as a cholesterol lowering agent. A two week dietary treatment in the Fischer 344 rat at doses ≥ 300 mg/kg/day resulted in the formation of multifocal hepatic microgranulomas. The lesion was associated with a mild (2/3X control) elevation in serum ALT levels and hepatic accumulation of ≥ 2000 μg LY295427 /g. Electron microscopy revealed the presence of crystalline inclusions in the secondary lysosomes and other membrane-bound spaces of the macrophages forming the microgranulomas and surrounding hepatocytes. Crystalline inclusions were also observed in hepatocytes of animals treated with 50 and 100 mg/kg/day LY295427, doses below the threshold for microgranuloma formation. A dose-response was evident at these doses in terms of liver concentration of LY295427 (23 ± 1 versus 90 ± 8 μg/g at 50 and 100 mg/kg/day, respectively), the number of hepatocytes containing crystalline inclusions, and the number of crystals per hepatocyte. These results suggest that dose-dependent accumulation of LY295427 is associated with the formation of crystalline inclusions in hepatocytes. At doses of ≥ 300 mg/kg/day, a critical hepatic concentration of LY295427 and/or number of intracellular crystals is achieved which results in hepatocellular injury (as indicated by serum ALT elevation), release of crystalline material into the extracellular space, and initiation of the inflammatory response leading to microgranuloma formation.

523 HIGH-DOSE INTOLERANCE TO THE ALZHEIMER'S THERAPEUTIC BESIPIRDINE HCl (HP 749) IN RATS IS BLOCKED BY PRE-TREATMENT WITH ALPHA$_1$ AND BETA$_1$ ADRENERGIC ANTAGONISTS

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HP 749, a potential Alzheimer's disease therapeutic, was previously associated with cardiomyopathies when tested at very high doses of 80 mg/kg/day in rats. Although this dosage is about 2000-fold higher than that required to improve learning and memory tasks in rodents, and about 100-fold higher than the maximum anticipated human dosage, it was of interest to determine whether the rodent myopathies were due to a direct effect of HP 749 on cardiomyocytes, or the were high-dose consequences of the known adrenergic effect of the drug. Thus, HP 749 was orally administered to 4 male SD rats at 80 mg/kg/day for 7 days. A similar group received pre-treatment (0.5 hr) with the alpha$_1$ (prazosin, 3 mg/kg/day p.o.) and beta$_1$ (atenolol, 2 mg/kg/day s.c.) adrenergic antagonists. Another group received the same adrenergic antagonists before and 5 hrs after treatments with HP 749 in order to prolong the antagonistic effects. A control group received the antagonists alone. Hemodynamic evaluations were conducted in animals receiving HP 749 with and without pretreatment, and histopathologic evaluation was performed on hearts of all dose groups. HP 749 at 80 mg/kg significantly increased mean arterial pressure by 33.6 ± 4.7%, decreased mean heart rate by 24.3 ± 4.1%, and resulted in severe ischemic cardiomyopathies (lethal in 2). Pre-treatment with the antagonists fully blocked the hemodynamic changes and lethal effects, leaving mild cardiac ischemia in just 3 of the 4 rats. The double administration of the antagonists fully blocked the cardiotoxicity. The high-dose rodent cardiomyopathies of HP 749 are thus attributable to the known adrenergic activity of the drug rather than an inherent direct toxicity. Ischemic cardiac pathology required prolonged, adrenergically mediated, hemodynamic changes.

524 SAFETY EVALUATION OF CEP-151 (rhIGF-I) IN CYNOMOLGUS MONKEYS

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CEP-151 (recombinant human insulin-like growth factor-I: rhIGF-I) is being developed by Cephalon, Inc. for use in the treatment of amyotrophic lateral sclerosis (ALS) and disorders of the peripheral nervous system. Safety assessment studies completed in cynomolgus monkeys to support clinical trials include single-dose and 7-day range-finding studies, and 1- and 6-month repeat-dose toxicity studies. Monkeys received either vehicle or rhIGF-I as a subcutaneous injection. Dose levels of CEP-151 for the single-dose study ranged from 0.17 to 4.0 mg/kg, and from 0.1 to 2.5 mg/kg/day in the 7-day, 1-month and 6-month studies. Dose-dependent decreases in blood glucose were observed and generally occurred within 1 hour postdose. Clinical signs consistent with hypoglycemia were observed in fasted animals at all doses and non-fasted animals at 2 and 2.5 mg/kg/day, and included hypoactivity, ataxia, prostration, tremors and/or convulsions. These signs were resolved with predose feeding and/or glucose treatment. In the multiple-dose studies, irritation (inflammation) at the site of injection was noted in all groups, including vehicle control. Compound-related increases in body weight were observed in the higher dose groups (0.5–2.5 mg/kg/day) in the 1-and 6-month studies. In the 6-month study, regenerative/hyperplastic renal tubular reactions (secondary to preexisting renal lesions) were noted in high-dose (2 mg/kg/day) females. Although antibody responses were apparent following treatment for 1 and 6 months, the positive titers were not associated with changes in the toxicologic profile or blood levels over time, indicative of a non-neutralizing nature. In conclusion, daily subcutaneous administration of CEP-151 to cynomolgus monkeys at doses up to 2.0 mg/kg for 6 months was well tolerated. Compound-induced findings were consistent with the "insulin-like" and anabolic properties of the protein.

525 RENAL ALTERATIONS AND LEUKOTRIENE ELIMINATION IN RATS ADMINISTERED AN IMMUNE SUPPRESSANT, CP-123,369

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CP-123,369, a macrocyclic immunosuppressant, was orally administered to Sprague-Dawley rats (10/sex/group) at 30, 100 and 300 mg/kg for one month. Two groups of animals received an equivalent volume (1 ml/100 g) of either (Group II) a cremophor/ethanol/water (20/10/70) vehicle or (Group I) a 0.9% saline control. Standard clinical observations and measurements were obtained including clinical, macro-, and microscopic pathology. A dose related decrease in body weight (males 6.5–11.3%; females 3.1–5.7%) and increase in food consumption (3.4–17.7%) were observed in CP-123,369 treated rats. Serum chemistry changes were limited to increased serum CHOL, TRIG, GLUC, BUN and CREAT (females). A dose-related incidence of renal tubular necrosis, regeneration, vacuolation and mineralization, pancreatic islet vacuolation and thymic atrophy were observed. Except for isolated renal tubular regeneration, similar lesions were absent from Group II rats. In a

separate experiment to evaluate the potential for leukotriene involvement in these effects, male rats received intravenously, in order: (1) glycerol formal (vehicle), (2) tritiated leukotriene (^{3}H-LTC$_4$; 2.4 nmol/kg) and after 90 min, (3) either vehicle (double control), CP-123,369 (20 mg/kg) or Cyclosporine A (CSA; 2, 5 or 20 mg/kg) and (4) ^{3}H-LTC$_4$. Bile and urine were collected continuously for 90 min postdosing, animals were necropsied and blood and tissue (liver, kidneys, heart, lungs) samples collected for measurement of ^{3}H-LTC$_4$ concentrations. CP-123,369 (20 mg/kg) decreased hepatic bile flow (14%) and LTC$_4$ elimination (30%) similar to 5 mg/kg CSA, and increased urine flow (39%) and renal LTC$_4$ elimination (56%) similar to 2 mg/kg CSA. Conversely, CSA reduced urine flow and LTC$_4$ elimination at 5 and 20 mg/kg, respectively. CP-123,369 did not alter LTC$_4$ renal or hepatobiliary retention, but did slightly increase cardiac and pulmonary retention. These findings are similar to those described previously for the related immune suppressants, CSA and Prograf (FK-506). However, the absence of hepatobiliary and renal leukotriene retention following acute CP-123,369 administration may reflect (1) a decreased toxic potential relative to CSA or (2) a need for subchronic administration (≥ 30 days) for leukotriene retention and resultant adverse renal effects.

526 13-WEEK ORAL TOXICITY STUDY OF 4-HPR WITH TAMOXIFEN IN RATS

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The toxicity of 4-hydroxyphenyl-all-*trans*-retinamide (4-HPR) with tamoxifen citrate (TX) was characterized in female Sprague-Dawley rats gavaged daily for 13 wk. Groups of 10 rats received the following daily doses (mg/kg) of 4-HPR and TX, respectively: 0:0 (vehicle control), 75:0, 0:7.5, 3:0.3, 15:1.5, and 75:7.5. The drugs were dissolved/suspended in an aqueous solution of 0.5% carboxymethylcellulose and 0.7% Tween® 80 Surfactant; the daily volume of administration was 20 mL/kg. Plasma levels of each drug during Wk 12 were correlated directly to the dose administered and was independent of the other drug. Beginning on Day 15, dose-related reduced growth rate was apparent in all groups receiving TX; 4-HPR alone had no effect on body weight. No treatment-related mortality, clinical signs, or hematologic effects were detected in any of the groups. TX administration resulted in decreases in group mean total protein, albumin and calcium; increases in alkaline phosphatase and phosphorus; and reductions in pituitary-to-brain weight ratios and ovary weights. Histologic changes were found in the ovaries, uterus, and vagina of all animals treated with TX. The effects of TX on the female rat reproductive tract were characterized by the presence of ovarian follicular cysts, lack of corpora lutea, and ovarian stromal atrophy; uterine endometrial gland atrophy and squamous metaplasia of both the endometrium and the endometrial glands; and increased numbers of goblet cells within the superficial vaginal epithelium. There were no definitive indications of synergistic or modulating effects of 4-HPR and TX on any of the parameters examined in this study. (Supported by NIH Contract No. N01-CN-95168-04)

527 DERMAL TOXICITY ASSOCIATED WITH PEGYLATED LIPOSOMAL DOXORUBICIN (DOXIL™) IN DOGS

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DOXIL (pegylated liposomal doxorubicin) is doxorubicin encapsulated in long-circulating liposomes. The most notable drug-related adverse effect in animals is reversible dermal lesions that develop primarily on the feet and legs with repeated doses. The lesions are similar to the dermal toxicity (hand-foot syndrome) seen in patients who receive continuous iv infusions of conventional doxorubicin. We examined the dermal effects of DOXIL in dogs using five different dosage regimens that varied dosage and dose frequency. Beagle dogs (2/sex/group) received 0.5, 1.0 or 2.0 mg/kg DOXIL from once every 7 to every 28 d for a total of 3 to 12 doses. The study was designed to achieve a cumulative dose of 6 mg/kg per animal in all treatment groups. Lesion severity was summarized by evaluation of 6 regions on each dog: the 4 limbs; the urogenital region-ventral body; and the head. Samples for clinical pathology and pharmacokinetics were collected periodically throughout the study. All dogs were necropsied on day 85. Myelosuppression was mild with all treatment regimens. Dermal lesions developed within 2 weeks of treatment in all groups, but resolved rapidly between doses or after the final dose. One female dog in the 2.0 mg/kg q^4 week dose group died on day 10 due to gastrointestinal toxicity, and the dose level of this group was reduced to 1.5 mg/kg. Higher dose intensities (higher dosages or lower dosages given frequently) caused more severe lesions, whereas increased interval between dosing or lower dosages minimized the severity of the dermal lesions. Cumulative dose was unrelated to lesion development. Repeated doses of 1.0 mg/kg and 1.5 mg/kg were not well tolerated at q2 and q4 weeks, respectively, and a dose of 0.5 mg/kg was not well tolerated at weekly intervals. Dogs that received 0.5 mg/kg at q2 or q4 weeks had less severe dermal lesions and greater lesion recovery than dogs on a weekly dose schedule. Lesion severity was greatest in the 1.5 mg/kg q4 week group, but lesions persisted longest in the 0.5 mg/kg weekly group. Thus, the severity of the dermal lesions was related to both dosage and dosing interval, but not to cumulative dose.

528 SINGLE-DOSE INTRAVENOUS TOXICITY OF CI-1010, A CYTOTOXIC RADIOSENSITIZING NITROIMIDAZOLE DERIVATIVE, IN RATS

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CI-1010, (R)-α-[[(2-bromoethyl)amino]methyl]-2-nitro-1H-imidazole -1-ethanol monohydrobromide, a hypoxic cell radiosensitizing anticancer agent, is a nitroimidazole analog designed with, in addition, a reactive alkylating functionality. The preclinical toxicity of CI-1010 was investigated in Wistar rats for up to 4 weeks following a single bolus injection at 0, 50, 100, 150, 225, or 350 mg/kg. Dose-limiting clinical toxicity was not apparent up to 350 mg/kg. Transient body weight loss up to 7% was observed at ≥ 100 mg/kg by Day 4. Dose-dependent hematologic effects at ≥ 150 mg/kg included 31 to 71% decreases in total leucocytes by Day 4, and 37 to 57% decreases in thrombocytes by Day 8. Flow cytometric evaluation of bone marrow on Day 4 showed dose-related decreases of 20 to 88% in total nucleated cell counts at ≥ 100 mg/kg with all cell types affected. All hematologic changes were reversed by Day 29. Histopathologic changes at Day 4 included lymphoid depletion (≥ 100 mg/kg), bone marrow hypocellularity (≥ 150 mg/kg), retinal degeneration (≥ 225 mg/kg) and testicular seminiferous tubular degeneration (≥ 150 mg/kg). Retinal changes were characterized by degeneration and/or absence of photoreceptor and outer nuclear layers. Ganglion cell layer vacuolation was evident by Day 29 at 350 mg/kg. Histopathologic changes in bone marrow and lymphoid tissues were reversible whereas the severity and/or incidence of testicular and retinal degeneration was progressive from Day 4 to 29.

529 TOXICITY OF ALBUTEROL DRY POWDER IN RATS, MONKEYS AND DOGS: A SPECIES COMPARISON

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Albuterol is a safe and quickly acting β_2-adrenergic agonist bronchodilator widely used by asthmatics. Even with its long history of safe use by asthmatics, concern exists regarding albuterol's potential cardiotoxicity. The aim of this study was to assess the toxicity of inhaled albuterol pure dry powder in rats, monkeys and dogs. All species were exposed to an aerosol of albuterol dry powder 1 hr/day, 7 days/wk for at least 2 wks. Plasma concentrations of albuterol confirmed systemic exposure. The daily inhaled dose received by the animals was calculated based on particle size data, published respiratory tract deposition data and daily measured test atmosphere concentrations of aerosolized albuterol. Multiples of the maximum daily clinical dose (15 μg/kg in a 70 kg human) were 0.25 – 2500 times in the rat, 9 – 100 times in the monkey and 0.5 – 90 times in the dog. Tachycardia and a transient hypokalemia occurred in rats at multiples of 1.5 times or greater of the maximum clinical dose. Increased absolute and relative heart weights also were observed in rats receiving multiples of 47 times or greater of the maximum human dose. In the absence of histopathologic findings, the changes in rat heart weight were considered a physiologic response to the increased heart rates. No test article-related findings were observed in the monkey. Dogs exhibited tachycardia and a transient hypokalemia at all doses tested as well as a slight to mild fibrosis of the papillary muscles of the heart at multiples of 19 times or greater than the human dose. The changes observed were due to the known pharmacologic action of B_2-adrenergic agonists. Due to the absence of heart lesions in the rat and monkey and to the unique sensitivity of B_2-adrenergic agonists in dogs, the heart lesions observed in dogs are not indicative of what is expected in the human population after repeated exposures of clinical doses of albuterol.

530 POPULATION PK/PD OF TISSUE FACTOR PATHWAY INHIBITOR (TFPI) IN CYNOMOLGUS MONKEYS DURING A 14-DAY TOXICITY STUDY

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The pharmacokinetics and pharmacodynamics (PK/PD) of recombinant human tissue factor pathway inhibitor (TFPI) were measured in 23 cynomolgus monkeys given doses of 20, 70, or 240 mg/kg/day by constant-rate IV infusion during a 14-day toxicity study. The change from baseline of prothrombin time (PT) was used for PD evaluation. Blood samples for PK/PD measurements were collected according to a semi-sparse schedule in order to limit the volume of blood removed and to reduce the number of venipunctures. Due to toxicities related to the anticoagulant properties of TFPI the high-dose was reduced to 150 mg/kg/day by Day 3 and eventually discontinued on Day 10. Based on a population PK/PD analysis, the TFPI after IV delivery was distributed in a central compartment of 520 ± 199 mL/kg and was distributed at steady-state in a volume of 5112 ± 628 mL/kg. The drug was eliminated from the central compartment with a clearance of 11.1 ± 0.88 mL/min/kg and distributed to the peripheral compartment with a clearance of 14.3 ± 3.08 mL/min/kg. For each ug/mL of TFPI in the plasma the prothrombin time was prolonged by 3.1 ± 0.264 sec. The PK/PD of TFPI were not significantly influenced by dose level, body weight, or sex. No time delay was observed between changes in TFPI plasma concentration and the PD response, nor did tolerance to TFPI develop with respect to changes in PT. Predicted steady-state plasma TFPI concentrations achieved with doses of 20, 70, and 240 mg/kg/day were 1.3, 4.4, and 15.0 μg/mL, respectively. The complexity of the blood collection and dosing schedules made a population-based analysis particularly useful in describing the PK/PD of TFPI in this study.

531 STABILITY, PALATABILITY AND TOXICITY OF HYDROGEN PEROXIDE ADMINISTERED TO MICE IN DRINKING WATER FOR 14 DAYS

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Previous drinking water studies on hydrogen peroxide (HP) did not establish stability of dosing solutions or quality assurance of data. The present study was undertaken to evaluate the stability, palatability and toxicity of HP when administered in drinking water to C57BL/6N (low catalase) mice. Palatability and toxicity were evaluated in mice (10/sex/group) given 0, 200, 1000, 3000 or 6000 ppm HP in distilled water for 14 days. Solutions were prepared fresh weekly. Stability of HP was verified analytically for 200 and 3000 ppm solutions offered to mice (>94.5% after 7 days). Palatability was affected at≥1000 ppm, based on depressed water consumption. No clinical signs of toxicity or body weight effects were observed at 3000 ppm or less. Mice given 6000 ppm had depressed body weights and food consumption. Histopathological evaluation revealed minimal/mild degenerative and regenerative alterations of the mucosa of the stomach and duodenum among the 3000 ppm and 6000 ppm animals. Based on the preceding observations the no observed adverse effect level for HP was 1000 ppm or 164 mg/kg for males and 198 mg/kg for females.

532 EXPERIMENTAL EVALUATION OF POTENTIAL ANTIDOTES FOR MONOCHLOROACETIC ACID (MCA) ACUTE INTOXICATION

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It has been hypothesised that the severe acute toxic effects induced by MCA after extensive accidental skin splashing are due to the accumulation of lactic acid in the cerebrospinal fluid (CSF), and that Phenobarbital (PB) and Dichloroacetic acid (DCA) may protect against MCA toxicity (Mitroka, 1989). The present study aims to verify the lactate hypothesis and the efficacy of three potential antidotes. Groups of 5 Sprague Dawley male rats were administered intravenously MCA (sodium salt) and an LD50 of 75 mg/kg was established. Hypokinesia, sedation, dyspnea, and suffocation were observed from 30 minutes after dosing, and coma and/or death from 1h15. Blood and CSF lactate was determined in groups of 5 rats at several time intervals after an i.v. injection of 80 mg MCA/kg. Lactate levels started to increase after 30– 40 minutes progressively until death, from 3 to ≥9 mmol/l in blood and from 5 to ≥ 16 mmol/l in CSF. The lactate level increased earlier in CSF than in blood and was paralleled by the development of the clinical signs. Groups of 6 rats were injected i.v. with 80 mg/kg MCA and, 15 minutes later, with PB, DCA (sodium salt) or 2-chloropropionic acid (2-CP, sodium salt). Eight-day survival rates were: MCA alone: 8%; 2-CP 45 mg/kg: 33%; 2-CP 90 mg/kg: 17%; PB 5 mg/kg: 50%; PB 40 mg/kg: 100%; DCA 50 mg/kg: 83%; DCA 110 mg/kg: 100%. Clinical signs induced by MCA were abolished after treatment with 110 mg DCA/kg. Blood lactate levels measured 90 minutes after the MCA injection were closed to the background control values in the groups treated with DCA and 40 mg PB/kg. DCA and PB, but 2-CP, appeared to be effective antidotes against MCA intoxication mediated by lactate accumulation. (Mitroka, J. G. [1989] Monochloroacetic acid lethality in the rat in relation to lactic acid accumulation in the cerebrospinal fluid, Ph.D. dissertation, Rutgers University, New Brunswick, NJ).

533 ASPIRATION TESTS IN AQUEOUS FOAM USING A BREATHING SIMULATOR

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Non-toxic stabilized aqueous foams are being developed by Sandia National Laboratories for the National Institute of Justice (NIJ). The potential for aspiration of aqueous foam during its use in the criminal justice prison systems, and the resulting adverse effects associated with complete immersion of an individual, is of major concern to the NIJ when examining the effectiveness and safety of using this technology as a Less-Than-Lethal Weapon. This study was designed to evaluate the amount of aqueous foam that might be aspirated by a healthy individual following total immersion in the foam. A T. W. Reed Breathing Simulator equipped with a 622 Silverman CAM was used to simulate the aspiration of an ammonium laureth sulfate aqueous foam generated at expansion ratios in the range of 500:1 to 1000:1. The results of this study indicate, that when breathing while immersed in the foam, an air pocket quickly forms around the nose and mouth. Maximum aspiration of foam occurs in the first 3–5 breaths with no additional foam aspirated upon continued exposure. The maximum amount of foam that was aspirated by the breathing simulator was found to be 9.4 gm for a one hour exposure to a 500:1 expansion ratio of this foam. Therefore, for its proposed use as a Less-Than-Lethal weapon in the prison systems, there is minimal aspiration risk associated with total immersion in this aqueous foam.

534 INTRAVENOUS (IV) SAFETY ASSESSMENT OF FS069: PERFLUOROPROPANE (PFP)-FILLED ALBUMINMICROSPHERE FOR CONTRAST ULTRASONOGRAPHY (US)

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FS069, an US diagnostic contrast agent is a suspension of PFP-filled albumin microspheres of 3.45–4.45μ average size and 6.30–8.85 X 10^8 microspheres/ml. Nonclinical safety assessment studies conducted: genetic toxicology, acute and subchronic (rat and dog), dog hemodynamics, rabbit irritation, *in-vitro* blood compatibility, rat radiolabeled PK, PFP PK in exhaled air and blood (rat), and teratology (Seg II) (rat and rabbit). Dosages used: 0.25, 2.5, 5.0, 10, 20, or 25 mL/kg. FS069 was non-genotoxic and non-irritantant, and was compatible with blood. FS069 did not elicit adverse effects in rat (acute) and dog (acute and subchronic) at dosages of up to 20 mL/kg and subchronic rat at 5.0 mL/kg. No changes in hemodynamic parameters were associated with FS069. Mortality in the acute rat (25 mL/kg) and the subchronic rat studies (20 and 10 mL/kg) resulted from passive pulmonary congestion, which did not appear to be longstanding, findings correlate with the fact that most of these rats did not display adverse signs prior to death. Mortality was probably a sequelae of the prolonged infusion and the tendency of FS069 microspheres to separate from suspension during injection. PFP was undetectable in blood and exhaled air within 1–3 minutes post injection. Conclusion: IV administration of FS069 at dosages and dose regimen used provided a large safety margin for the estimated clinical efficacious dosages for diagnostic ultrasound imaging.

535 TOXICOLOGICAL EVALUATION OF 14 DAY CONTINUOUS INFUSION OF RECOMBINANT HUMAN TISSUE FACTOR PATHWAY INHIBITOR (TFPI) IN THE CYNOMOLGUS MONKEY

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Tissue factor expression plays a central role in the pathogenesis of sepsis and cardiovascular disease. For this reason, TFPI is being developed as a therapeutic agent for these indications. The toxicological and pharmacokinetic profile of TFPI administered by continuous infusion for up to 14 days with continuing evaluation in a reversal interval was determined in the cynomolgus monkey. As expected TFPI caused a dose related prolongation in prothrombin time (PT) and activated partial thromboplastin time, which, in the high dose group, was associated with the potential for hemorrhagic diathesis. However, the only histopathological correlates noted at any dose were hematomas at the site of femoral vein venipuncture. The pharmacological and toxicological effects of TFPI were rapidly reversed upon cessation of infusion. The relationship of PT to TFPI plasma concentration was linear with a positive slope of 3.1 sec per μg/mL TFPI. Mild to moderate antibody titers to TFPI were correlated with dose and duration of dosing and were first detected on day 10 in the high dose group (2/6 animals). Aside from mild changes in plasma triglyceride and cholesterol levels, the toxicological correlates of TFPI administration are related primarily to its mechanism of action. At doses associated with efficacy in preclinical models of sepsis and cardiovascular disease TFPI is safe.

536 SAFETY PHARMACOLOGY EVALUATION OF THE PHOSPHODIESTERASE TYPE IV INHIBITOR BRL 61063

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The effects of single oral doses of BRL 61063 on cardiovascular, renal and respiratory functions were evaluated in cynomolgus monkeys or Sprague-Dawley rats. Cardiovascular changes were monitored in conscious monkeys continuously for 24 hours post-dose. A transient tachycardia (heart rate increases of +25%) with no change in blood pressure was evident between 40–90 minutes following single oral doses of 0.1 to 3 mg/kg. Cardiovascular and renal functions were evaluated in conscious rats continuously for 3 hours after dosing and at 48–51 hours post-dose. A single oral dose of 3 mg/kg produced increases in renal vascular resistance and urine osmolarity (+220 and +14%, respectively) and reductions in glomerular filtration rate, renal blood flow and urine volume (-66, -62 and -41%, respectively) during the 0–3 hour post-dose period. Tachycardia (heart rate increases of +40%) was also evident at this time. All values returned to normal at 48–51 hours post-dose, and there were no effects at a dose of 0.3 mg/kg. Respiratory parameters were monitored in conscious rats at 4, 24 and 48 hours post-dose. A single oral dose of 3 mg/kg produced respiratory depression at 4 and 24 hours post-dose. This depression was associated with 16–38% decreases in tidal volume and mean inspiratory flow (respiratory drive). These changes returned toward normal by 48 hours post-dose and there were no changes at a dose of 0.3 mg/kg. There were no changes in the mechanical properties of the lung. In summary, a single oral dose of 3 mg/kg BRL 61063 produced acute changes in cardiovascular and renal functions and respiratory depression that persisted for 24 hours. Transient tachycardia was observed at doses ≥ 0.1 mg/kg.

537 INTRADERMAL TOXICITY OF RECOMBINANT-METHIONYL HUMAN STEM CELL FACTOR (r-metHuSCF) IN BABOONS

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In previous *in vivo* and *in vitro* studies, r-metHuSCF has been shown to stimulate melanocytes and might therefore be useful in the treatment of pigmentation disorders. The current study investigated the potential toxicity associated with administration of r-metHuSCF via the intradermal clinical route. The baboon was selected based on previous studies in which the model has been shown to be the most sensitive to the pharmacological actions of r-metHuSCF. Ten adult male baboons (1-3/group) were administered 0 (vehicle), 10, 100 or 600 μg/kg of rmetHuSCF intradermally at a dose volume of 0.5 ml/kg. Animals received a single intradermal injection, followed by a seven day washout period, and a cycle of seven consecutive daily repeated-intrademal injections. Biopsy specimens were collected at the site of injection and at a contralateral control site before and after each treatment cycle for histologic evaluation. No mortality or overt signs of toxicity were observed. A very slight erythema was observed intermittently at the injection site in one animal in each r-metHuSCF treated group. While within normal range for the species, a decreased (22-60%) leukocyte count, primarily attributed to neutrophils, was observed as compared to baseline in all r-metHuSCF treated animals seven days following the initial single intradermal dose. No other biologically significant changes in clinical pathology parameters were observed. Microscopically, a clear dose-dependent increase in mast cells was evident after the seven day repeated treatment cycle but not after a single intradermal dose. A qualitative assessment of dermal melanocytes indicated an increase in melanocytes in specimens corresponding to the injected sites as compared to contralateral control sites, although a dose relationship was not observed. This study suggests that intradermal injection of r-metHuSCF is tolerated at doses which are associated with an increase in melanocytes.

538 A SALT SWITCH: COMPARISON OF OCTREOTIDE ACETATE (SANDOSTATIN®) VERSUS OCTREOTIDE PAMOATE TOXICITY IN RATS

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The purpose of this study was to compare the toxicological effects of octreotide acetate (o-ac), octreotide pamoate (o-pa), and sodium pamoate (Na-pa) following daily subcutaneous administration to Sprague-Dawley rats for 4 weeks. Rats were administered vehicle, o-ac (4 mg/kg/day), o-pa (4 mg/kg/day), or Na-pa (4.6 mg/kg/day) using a dose volume of 5 mL/kg. In all groups, clinical signs were limited to the injection-site area and consisted of wounds and scabs. These signs were noted primarily in the octreotide-treated groups and to a lesser extent in the Na-pa group. Compared to the control group, octreotide treatment resulted in lower food consumption and body weights; there were no differences between the octreotide-treated groups. There was no toxicologically relevant clinical pathology finding. Postmortem findings were limited to the injection-site area and at necropsy included thickened skin and hemorrhage. Microscopically, injection-site lesions were characterized by connective tissue proliferation and chronic inflammatory cell infiltration suggestive of a generalized chronic response to injury. Octreotide-treated groups had slightly more inflammatory changes compared to the control or Na-pa groups; there were no differences between the octreotide-treated groups. Injection-site lesions in the Na-pa treated animals were comparable to controls. Toxicokinetic evaluation on Day 1 indicated that the AUC values for octreotide base were comparable between the 4 mg/kg/day o-ac and o-pa groups. On Day 28, the AUC for the o-pa group was approximately 40% lower than that of the o-ac group; however, given the variance associated with these values, this apparent difference is probably not significant. In summary, the acetate and pamoate salt forms of the octreotide base and Na-pa were well-tolerated by rats and there were no differences in the toxicity of the two salt forms.

539 A 28-DAY SUBCUTANEOUS TOXICITY STUDY OF RECOMBINANT-METHIONYL HUMAN NEUROTROPHIN-3 (r-metHuNT-3) IN RHESUS MONKEYS: POSSIBLE HORMONAL EFFECTS

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Data derived from preclinical animal models have suggested that r-metHuNT-3 may have potential in the treatment of peripheral sensory neuropathies. Therefore we conducted a 28-day subcutaneous toxicity study in rhesus monkeys in the dose range of 1–30 mg/kg/day. Significant increases in body weight gain were noted in all treated animals, while food consumption was affected differently between males (increased) and females (decreased) treated with r-metHuNT-3. There were no biologically relevant changes in clinical chemistry or hematology. Microscopic changes (maturation of testes, prostate) and effects on organ weights (testes, prostate, ovaries, uterus, pituitary) were noted. These observations were indicative of endocrine effects on these selected target organs. Leutinizing hormone (LH) and testosterone were subsequently measured. Increases in LH in females and increases in both LH and testosterone in males were seen as early as one week after dosing at 5 and 30mg/kg/day. Following a one-month recovery period these effects were shown to be reversible in the high dose group. These findings suggest that

specific target organ weight changes and maturation processes observed in animals dosed with r-metHuNT-3 may be related directly or secondarily to specific endocrine effects.

540 A NINETY DAY ORAL TOXICITY STUDY OF R-ALBUTEROL IN SPRAGUE-DAWLEY RATS

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Albuterol is a selective β_2 adrenergic agonist that is administered either by inhalation or orally for the symptomatic relief of bronchospasm. However, the major adverse effect of such β-adrenergic agonists is the induction of a bronchial hyperreactivity, which is not mediated by via β receptors. This undesirable effect is believed to be caused by the S-isomer of albuterol, with the therapeutic activity residing in the R-isomer. In order to assess the subchronic toxicity of this material, R-albuterol was orally administered for 90 days to two groups of 40 Sprague-Dawley rats (20/sex/group) at dose levels of 2.5 and 12.5 mg/kg/day and two groups of 60 Sprague-Dawley rats (30/sex/group) at 0 and 25 mg/kg/day. For comparison, R,S-albuterol was orally administered to 60 rats (30/sex) at a dose level of 50 mg/kg/day. No adverse test article related effects were found relative to body weights, food consumption values or urinalyses. Similarly, there were no adverse ophthalmological or gross necropsy findings. However, statistically significant decreases were observed in group mean serum glucose values for all test article treated groups. An interesting finding was a statistically significant increase in absolute and relative spleen weights for both RS- and R-albuterol. Histopathological evaluation of the spleens from treated animals revealed reversible microscopic lesions of capsular thickening, development of capsular cysts and mild to moderate multifocal capsulitis. Based upon the results of this work, a NOEL for R-albuterol of < 2.5 mg/kg/day was recommended.

541 SAFETY ASSESSMENT OF RECOMBINANT HUMAN INTERLEUKIN-10 (rhuIL-10) BASED ON TOXICOKINETIC EVALUATIONS IN CYNOMOLGUS MONKEYS

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The toxicokinetics of rhuIL-10 were evaluated in cynomolgus monkeys following intravenous (IV) and subcutaneous (SC) administrations to support the preclinical safety program in establishing clinical safety margins. Three monkeys/sex/dose were administered rhuIL-10 at doses of 5, 15, 45 μg/kg (IV) or 5, 25, 75 μg/kg (SC) daily for 30 days. Serum samples were collected and pharmacokinetic (PK) parameters evaluated on Days 4, 15 and 30. Serum rhuIL-10 levels were quantitated by an ELISA and bioassay. The presence of anti-rhuIL-10 antibodies (Ab) and serum neutralizing factor (SNF) activity were determined by an ELISA and bioassay, respectively. PK assessments were based on ELISA data. Systemic exposure to rhuIL-10 was dose-related and sex independent by both routes of administration. Systemic exposure (IV) was similar at all intervals indicating no accumulation of rhuIL-10. Accumulation following SC dosing was not assessed due to the presence of non-specific inhibitory factors (ELISA) which precluded PK evaluations on Days 15 and 30. The elimination half-life (IV) was 1.5 to 2 hours. rhuIL-10 was absorbed and eliminated rapidly following SC administration. Peak serum concentrations were observed 2 to 4 hours post-dose and the elimination half-life was ~2 hours. Bioassay data suggested a dose-related increase of biological activity at all intervals by both dose routes. There were both time- and dose-dependent increases of anti-rhuIL-10 Ab (IV, SC) following 15 and 30 days of dosing which persisted during a one month recovery period. No evidence of SNF activity was observed to correlate with the detection of anti-rhuIL-10 Ab. Comparison of the systemic exposure between the No Observed Adverse Effect Levels established in monkeys and the proposed clinical doses in humans has demonstrated a 0.3- to 14-fold (IV) and 0.5- to 29-fold (SC) safety margin.

542 SEX SPECIFIC HEMORRHAGIC SYNDROME SECONDARY TO VITAMIN K DEPLETION IN CHOLESTAGEL™ TREATED RATS

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CholestaGel™ is a novel bile acid sequestrant with ultra high binding affinity for primary conjugated bile acids. Binding of bile acids can disrupt micelle formation resulting in decreased absorption of fat soluble vitamins. Ten Sprague Dawley rats per sex per group received CholestaGel™ in the diet at doses of 0, 400, 1200 or 3600 mg/kg/day for 28 consecutive days. Eight male high dose animals died or were moribund sacrificed during the study. Based on macro-and microscopic evidence of hemorrhage in a variety of tissues and supporting hematologic data suggesting anemia death was attributed to massive hemorrhage. Hypoprothrombonemia and decreases in fat soluble vitamins D, E and K were also observed in the male rats. No clinical signs of toxicity, and no macro- or microscopic findings were observed in CholestaGel™-treated females or intermediate and low dose males. In order to further elucidate the mechanism of this hemorrhagic syndrome, Four Sprague-Dawley rats per sex per group were administered CholestaGel™ in their diet at a dose of 9000 mg/kg/day for three weeks and injected once per week subcutaneously into the intrascapular fat pad with vitamin K_1 (0.1 mL/kg in safflower oil) at doses of 0, 0.5, 1.5 or 5.0 mg/kg. Control animals (no vitamin K_1 injections) had a mean survival of 9 days. Animals receiving 0.5 mg/kg vitamin K_1 had a mean survival of 15.5 days; animals receiving 1.5 mg/kg vitamin K_1 had a mean survival of 18.75 days with three still alive at study termination. All of the animals administered 5 mg/kg vitamin K_1 were alive at study termination with no clinical signs of toxicity. Sex specific hemorrhagic syndrome secondary to vitamin K depletion has been previously reported in rats. These data indicate that CholestaGel™ induced a hemorrhagic syndrome by preventing absorption of dietary vitamin K.

543 HUMAN HEALTH HAZARD IDENTIFICATION FOR OIL PRODUCTION AND REFINERY CHEMICALS CONTAINING PHENOL/FORMALDEHYDE RESINS

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Phenol/Formaldehyde (P/F) resins are widely used oil production and refinery chemicals as intermediates and components of demulsifiers, corrosion inhibitors, and emulsifying agents. Two P/F resins were evaluated for their acute and subacute toxicity in rats and rabbits and for their potential to induce dermal sensitization in guinea pigs. One P/F resin formulation contained 11% xylene. The other P/F resin formulation contained 44.3% of a solvent composed of xylene (83%) and ethylbenzene (17%). All rats survived oral gavage treatment with 5.0 g/kg bw of both P/F resins. All rabbits exposed dermally to 10 g/kg bw of both P/F resins survived. No unusual clinical observations were noted and body weight gains were normal. Dermal irritation was slight to moderate for the P/F resin containing 11% xylene and moderate to severe for the P/F resin in the xylene/ethylbenzene solvent. Neither of the P/F resins induced skin sensitization in the guinea pig. In rabbit 28-day dermal toxicity studies, there were no statistically significant and/or biologically significant treatment-related effects on clinical observations, body weight, food consumption, organ weights, hematology values, gross postmortem findings, and histopathology up to and including the high dose of 2 ml/kg of a 10% preparation (w/v). The results suggest that the P/F resins evaluated are not significant hazards for the health effects evaluated.

544 ACUTE AND SUBCHRONIC ORAL TOXICITY OF THE ANTICYANIDE DRUG WR242511 TARTRATE

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These studies examined the acute oral toxicity of WR242511 tartrate in CD® rats and CD-1 mice and the subchronic oral toxicity of WR242511 tartrate in CD® rats and Beagle dogs when administered daily for 13 weeks and 4 weeks, respectively. The acute oral LD50 of WR242511 in male rats was approximately eight-fold lower than in female rats (males; 16.3 mg base/kg and females; 135 mg base/kg). A sex-related difference in the acute oral LD50 was not observed in mice (males; 23.0 mg base/kg and females; 22.45 mg base/kg) Dose levels for the subchronic oral toxicity studies in the rat by gavage were 0, 0.5, 1.5 and 4.5 mg base/kg/day and in dogs by capsule were 0, 0.1, 0.3 and 1.0 mg base/kg/day. The primary treatment-related effects in both species included hemolytic anemia, thrombocytopenia and alveolar histiocytosis. Compensatory responses to the anemia included reticulocytosis, splenic hemosiderosis (rats), extramedullary hematopoiesis (dogs), and bone marrow hyperplasia (dogs). Hepatotoxicity, manifested by hepatocyte degeneration and necrosis accompanied by serum chemistry changes, was observed in male, but not female rats, and may have contributed to the early death of

seven of ten high dose males. Significant methemoglobinemia, the desired pharmacologic effect, was observed in high and mid dose animals of both species and was manifested by clinical signs of cyanosis in dogs. Generalized leukocytosis and thymic depletion in rats were considered secondary to stress. Alveolar histiocytosis, thrombocytopenia, and anemia were seen in rats at all three WR242511 dose levels. Toxic effects in dogs were primarily limited to the high dose level although thrombocytopenia and mild signs of cyanosis were also seen in mid dose animals. Supported by USAMRMC Contract No. DAMD17-92-C-2001.

545 HEALTH IMPACT FROM CHEMICAL COMPONENTS OF CELLULOSE INSULATION

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An evaluation of cellulose insulation was conducted to identify potentially toxic components. Samples of insulation materials untreated, and treated with 2 fire retardents were analyzed for volatile and extractable constituents. Volatile constituents were sampled by packing aliquots into VOA-vials, holding them at room temperature or heating to 90°C for at least one hour, and analyzing the headspace air by GC-MS. Extractable constituents were sampled by packing aliquots into VOA-vials, extracting with methylene chloride, evaporating to dryness, redissolving in methanol, and analyzing the concentrates by GC-MS. Because of the propensity for formaldehyde to polymerize in the gas chromatograph, a separate analysis was done by HPLC using DNPH. Analyses of headspace air at room temperature indicated no detectable quantities of volatile constituents. Analysis of headspace air at 90°C showed the occasional presence of traces (1–100 μg/L) of aliphatic and aromatic hydrocarbons and higher aldehydes The range of detected trace organics in extracts of insulation samples was 0.4–40 ppm. The highest peaks matched the mass spectra of an octadecadienoic acid methyl ester, a substituted cyclohexenecarboxylic acid, phthalate plasticisers, BHT, and methyl palmitate. Headspace samples were obtained at a temperature exceeding the upper limit to which properly installed insulation would be exposed through use as a building material. Even at 90°C, dilution of the relatively small amount of air entering a building from the insulation space with the air volume of the building would result in maximal possible concentrations in the low ppb range. There was no formaldehyde detected in any of samples. No negative health impact is predicted at these levels.

546 SAFETY EVALUATION OF DepoCyt™ BY INTRATHECAL ADMINISTRATION IN RHESUS MONKEYS

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DepoCyt®, a sustained-release formulation of cytarabine encapsulated in DepoFoam was administered intrathecally (IT) to Rhesus monkeys for 4 cycles (1 dose/14-day cycle). Preliminary studies in monkeys established the MTD and toxicokinetics of IT cytarabine. For the main study, 9M, 9F were divided into four groups: 1M, 1F (saline); 3M, 3F (vehicle); 2M, 2F (5 mg/animal); 3M, 3F (10 mg/animal). Doses were administered IT by infusion (~ 15 min) via a port-catheter system at L3 - L6. Dexamethasone (1 mg) was also given during each cycle to control inflammatory responses to cytarabine. Animals were sacrificed 14 days after the fourth dose (end of cycle 4) except for 1M, 1F in the vehicle and 10 mg groups which were retained to evaluate recovery or long-term effects. Criteria evaluated for treatment effect included clinical and neurological evaluation, body weight, food consumption, ophthalmology, hematology, clinical chemistries, urine analysis, ECG, blood pressure, CSF analysis, macroscopic and microscopic evaluations - with special emphasis on neuropathology. CSF was analyzed for cytarabine content by HPLC and LC/MS/MS methodology. Cytarabine-related toxicity evident in both 5 and 10 mg groups (usually dose related) was limited to reduced BW and FC as well as increased WBC and CSF changes in electrolytes and total protein. Two animals at 10 mg developed stiff backs and were recumbent but recovered after dexamethasone treatment. Neuropathology findings were toxicologically insignificant. Cytarabine was shown to be present in CSF in some animals 14 days after the last dose. This study substantiates the safety of intrathecal DepoCyt® in a regimen similar to human anticancer therapy (neoplastic meningitis) but at much higher dose levels.

547 COMPARATIVE DERMAL SUBCHRONIC TOXICITY STUDIES OF DIISOPROPYLCARBODIIMIDE (DIC) AND DICYCLOHEXYLCARBODIIMIDE (DCC) IN F344 RATS AND B6C3F$_1$ MICE

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DIC (CAS No. 693-13-02) and DCC (CAS No. 538-75-0) were evaluated, as representative carbodiimides, for toxicologic effects because of their use as stabilizing, coupling, and condensing agents in the chemical, pharmaceutical and biopharmaceutical industries; reported occupational instances of skin and eye irritation, allergenicity and sensitization and eye effects; and because of the potential of the alkylcarbodiimides to serve as potential mutagens and carcinogens. DIC and DCC were administered to 10 animals/sex/species in ethanol at doses of 0, 10, 20, 40, 80 and 160 mg/kg and 0, 0.75, 1.5, 3, 6 and 12 mg/kg in rats, respectively, and 0, 17.5, 35, 70, 140 and 280 mg/kg and 0, 1.5, 3, 6, 12 and 24 mg/kg in mice respectively, for 13 weeks. Clinical pathology samples were collected at 3, 22 and/or 92 days. Chemical-related deaths occurred in a dose-related fashion at the upper end of the range for both chemicals and species. Body weight effects extended to doses at the NOEL for mortality. Clinical signs of toxicity, which were dose-related in terms of severity and incidence, included irritation at the application site and indications of neurotoxicity for both chemicals (abnormal breathing, tremors, seizures, coma, ataxia). For DIC clinical pathology indicators of hepatoxicity were noted in rats, while DCC hepatoxicity indicators and anemia were noted in rats and microcytic anemia in mice. Histopathology indicated epidermal hyperplasia and inflammation at the dermal application site as an effect of DIC, with the mice more sensitive than rats and a rat specific edema, congestion and hemorrhage of the brain. DCC caused epidermal necrosis, hyperplasia and inflammation at the application site in both species, bone marrow and spleen effects in the rat and preputial gland and brain effects in mice. DCC was the more potent toxicant since effects were more severe and noted at lower doses (mg and moles/kg). (Supported by NIEHS Contract N01-ES-15319).

548 PATHOLOGIC FINDINGS IN CYNOMOLGUS MONKEYS ADMINISTERED IL-4

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Interleukin 4 (IL-4) has a wide range of immunomodulatory activities, including increasing endothelial adhesiveness for leukocytes by increasing VCAM-1 expression, and enhancing mast cell proliferation. Recombinant human IL-4 (rhuIL-4) was administered daily by subcutaneous injection to cynomolgus monkeys (*Macaca fascicularis*) at doses of 0, 5, 25, and 50 μg/kg for 1 month, or 0, 1, 5, and 30 μg/kg for 6 months. Antemortem and clinicopathologic findings are described in a companion poster (Snyder et al). Significant findings were seen in the cardiovascular and hepatobiliary systems, and in the spleen. Scattered vasculitis, perivasculitis, and thrombosis were seen in multiple tissues. The vasculitis often included eosinophils. Scattered eosinophils, or mononuclear cell infiltrates, were observed in the heart. Myocardial necrosis or fibrosis were also present in a few monkeys. Increased numbers of mast cells were seen in the heart and liver. Other hepatic changes consisted of sinusoidal cell hyperplasia, sinusoidal fibrosis, necrosis, suppurative hepatitis, mononuclear cell hepatitis, biliary epithelial hyperplasia, and gall bladder wall edema. Splenomegaly, associated with alterations in the red pulp, was observed. Splenic capsular fibrosis/capsulitis was also seen. The incidence and severity of findings generally related to dose and duration of rhuIL-4 administration, and many findings were attributed to the known biological activities of IL-4.

549 EFFECTS OF SUBCUTANEOUS rhuIL-4 IN CYNOMOLGUS MONKEYS

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The effects of recombinant human interleukin-4 (rhuIL-4) administered as daily subcutaneous injections to cynomolgus monkeys were studied at doses of 0, 5, 25 or 50 μg/kg for one month or at 0, 1, 5 or 30 μg/kg for six months. Serum neutralizing factor activity and/or antibodies to IL-4 were dose-and time-related. The no-effect dose for systemic toxicity was identified as 1 μg/kg/day for six-months. The one-month non-lethal dose was ≥25 but <50 μg/kg/day. Two male monkeys dosed at 30 μ/kg died after six months with hepatic necrosis complicated by disseminated intravascular coagulation (DIC). Biologic and toxicologic effects in both studies were generally dose

and time-related and included findings in the the cardiovascular and hepatobiliary systems, spleen and injection site. Pathologic findings are described in a companion poster (Leach et al). Clinical pathologic changes common to both studies at ≥25 μ/kg included leukocytosis, lymphocytosis, thrombocytopenia, prolonged partial thromboplastin and prothrombin times, increased AST and ALT values and increased triglycerides. Clinical signs were generally dose-related and included swellings at and/or distant from the injection site and diarrhea, scant feces and emesis. Body weight losses, food consumption reductions and/or clinical signs associated with a moribund condition occurred at doses ≥25 μg/kg. Multiple ECGs were not predictive of cardiac histopathologic changes, and no effects or only a few sporadic spontaneous arrhythmias were recorded until week 27. At week 27 three monkeys dosed with 30 μg/kg had minor QT interval lengthening. Physical examinations revealed no changes in ophthalmology, body temperature, blood pressure or respiratory or heart rates.

550 CHRONIC TOXICITY ASSESSMENT OF RECOMBINANT HUMAN BASIC FIBROBLAST GROWTH FACTOR IN CYNOMOLGUS MONKEYS

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Recombinant growth factors are currently being developed to provide healing of a variety of wound types (e.g. pressure sores). Recombinant human basic fibroblast growth factor, rhbFGF, is a single chain polypeptide derived via DNA recombinant techniques from Escherichia coli. To evaluate the chronic toxicity of rhbFGF, 48 kennel-raised cynomolgus monkeys received subcutaneous administrations of 0, 5, 15, and 45 μg/kg daily for 26 consecutive weeks. Daily injections were given via 4 sites in the dorsal aspect at dose volumes of 0.1 or 0.3 mL/kg. Physiological and laboratory investigations were undertaken at regular intervals. Blood samples for antigenicity and proof of absorption investigations were obtained at regular intervals during the course of the study. At the end of the 26 week treatment period, 2 animals/sex were retained for a 13 week recovery period. Aside from subcutaneous thickening at the injections sites, there were no clinical reactions to treatment. One test material related death in a high dose male was caused by a moderate protein losing nephropathy. Generalized subcutaneous edema was observed in this animal prior to death. No test material related signs of toxicity occurred at the low or mid dose levels. The no toxic effect dose was 15 ug/kg/day.

551 TRIETHYLENE GLYCOL (TEG): SUBCHRONIC PERORAL TOXICITY STUDY IN RATS

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Ethylene and diethylene glycol are known to cause hepatorenal injury by acute and repeated peroral dosing. TEG (CASRN 112-27-6) was investigated to determine its potential to cause hepatorenal injury when given perorally. Acute studies showed a peroral dose of 16.0 ml kg^{-1} to 5 male and 5 female albino rats did not produce mortality or pathology. A 2-week dose-range finding study with Fischer 344 rats receiving TEG at 0, 10000, 20000 or 50000 ppm in the diet resulted only in increased urine volume with decreased pH and triple phosphate. A 90 day study was conducted in the same species at the same dosages; equivalent to 748, 1522 and 3849 mg TEG/kg/day with males and 848, 1699 and 4360 mg/kg/day with females. There were no signs or effects on food consumption, clinical chemistry, necropsy, or histology. Body weight was reduced in high dose animals. Hematology showed (males) decreased erythrocyte count and hematocrit (mid and high doses) and increased MCV with decreased hemoglobin (high dose). Increased urine volume with decreased pH, and increased relative kidney weight, were measured without biochemical or histological evidence for renal injury. The urinary changes probably relate to excretion of the high TEG doses (or metabolites). Unlike lower MW homologues, TEG did not produce hepatorenal injury.

552 SCREENING OF QUINOLONE ANTIBIOTICS USING A BALB/C 3T3 NEUTRAL RED UPTAKE PHOTOTOXICITY ASSAY

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Balb/c 3T3 mouse fibroblast cells were exposed to 8 concentrations of either ciprofloxacin, lomefloxacin, norfloxacin, ofloxacin, or 7-(3-amino-1-pyrrolidinyl)-quinolones: clinafloxacin (8-chloro), CI-938 (8-fluoro), PD 124816 (5-amino, 8-fluoro), or PD 135044 (8-methoxy) in phosphate-buffered saline for 1 h at 37 °C. Cells were then exposed to either UVA light (320–400 nm, 5 J/cm^2, phototoxicity) or darkness (cytotoxicity) for 50 min. at ~25 °C. The cells were then incubated at 37°C for 24 h in drug-free growth media. Neutral red (NR) media was added after 24 h and incubation continued for 3 h. The cells were then washed and lysed with fixative. Cell viability was determined by comparing NR absorption of controls to that of treated cells. Inhibitory concentrations where viability was 50% of control (IC_{50}) after quinolone + UVA exposure were 600, 60, 40, 38, 15, 9, 3, and 2 μg/ml for PD 135044, norfloxacin, ofloxacin, ciprofloxacin, lomefloxacin, clinafloxacin, CI-938 and PD 124816, respectively. The IC_{50}s for cytotoxicity were >300 μg/ml for all compounds tested. The slopes of the concentration-viability curves were similar for all compounds tested. Phototoxicity rank order of most compounds was comparable to that from the CD-1 depilated mouse model and/or clinical studies, as PD 135044, norfloxacin, ofloxacin and ciprofloxacin are less and CI-938 is more phototoxic than lomefloxacin. However, the rank order of clinafloxacin, lomefloxacin and PD 124816 in vitro differs from that in mice, as clinafloxacin and PD 124816 are less phototoxic than lomefloxacin in vivo. It appears, that this assay can be used to provide a first approximation of the relative phototoxicity of quinolones: low (PD 135044), medium (ciprofloxacin, norfloxacin and ofloxacin) and high (lomefloxacin, clinafloxacin, CI-938 and PD 124816), but an vivo model which incorporates metabolism and distributional factors is needed to more accurately predict clinical phototoxicity.

553 ENHANCED CYTOTOXICITY OF CYCLOPHOSPHAMIDE (CPA) BY DEXRAZOXANE (DZA) IN MICE AND RATS

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DZR is a derivative of EDTA which has been shown to ameliorate doxorubicin (DOX) -induced cardiomyopathy in animals and humans. DZR also inhibits topoisomerase II and causes G_2/M blockade. Since DOX is often given with CPA as part of cancer chemotherapy regimens, studies were conducted in mice and rats to determine whether DZR affects the efficacy or toxicity of CPA. In mice bearing the experimental tumors, P388, L1210, Gross leukemia or Lewis lung carcinoma, DZR had little or no antitumor activity. CPA was active in these models and when combined with DZR, there was a marked enhancement of efficacy with cures in P388, Gross leukemia and Lewis lung carcinoma. In rats, doses of DZR up to 320 mg/kg, which were well tolerated when given alone, caused an increase in CPA-induced lethality and severe leukopenia. Interactions of this magnitude were not observed when DZR was administered with anthracyclines or other cytotoxic drugs used in cancer chemotherapy. These results suggest that there is a synergistic cytotoxic effect when DZR is combined with CPA.

554 SAFETY ASSESSMENT OF A NOVEL INTERLEUKIN-3 RECEPTOR AGONIST IN THE CYNOMOLGUS MONKEY

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Studies were conducted in the cynomolgus monkey to determine adverse effects associated with SC-55494, an interleukin-3 (IL-3) receptor agonist under development as a bone marrow progenitor cell stimulant. SC-55494 was administered by subcutaneous injection at doses of 5, 15 and 50 μg/kg/day for up to 30 days. In addition, clinically relevant doses of G-CSF or GM-CSF (10 μg/kg/day) were co-administered with 15 μg SC-55494/kg/day by subcutaneous injection. Parameters examined were clinical signs, clinical pathology, antibody titers, acute phase proteins and cytokines, gross and histopathology. Pharmacologic effects of increased peripheral blood leukocyte counts were observed at all doses of SC-55494 due to increases in neutrophil, lymphocyte and basophil counts (high dose) and eosinophil counts (all doses). Increased peripheral blood leukocyte counts were also observed in monkeys co-administered SC-55494 with G-CSF or GM-CSF. A synergistic effect was evident on this parameter between SC-55494 and G-CSF. Increases in platelet counts occurred at all doses in SC-55494-treated male monkeys with an additive effect apparent in male monkeys co-administered GM-CSF. Injection site edema was transient, occurred in the high-dose SC-55494-treated monkeys during the first 2 weeks, and was observed in all test article-treated monkeys during the last 2 study weeks. Antibody titers to SC-55494 were present by day 16 and during the reversal period. All clinical signs and clinical pathology changes were reversible. In summary, SC-55494 at

exaggerated doses was clearly tolerated in a pharmacologically relevant test species.

555 PHARMACODYNAMIC ASSESSMENT OF RECOMBINANT HUMAN TISSUE FACTOR PATHWAY INHIBITOR (TFPI) IN THE CONSCIOUS BABOON

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Sponsor: G C Haggerty

TFPI is an endogenous inhibitor of tissue factor initiated blood coagulation. Since this pathway is of critical importance in cardiovascular disease and sepsis, TFPI is being developed for these indications. The pharmacological and potential toxicological effects of a 12 hour continuous infusion of TFPI were investigated in female baboons at two doses. The low dose (1 mg/kg loading dose, 9.5 μg/kg/min) was intended to reproduce the efficacious dose previously determined in a primate model of sepsis. At a five-fold exaggeration (5 mg/kg loading dose, 47.5 μg/kg/min) of that dose mean peak plasma levels were proportional to dose and were 1130 ± 330 and 5180 ± 850 ng/mL, respectively. Plasma concentrations appeared to be at steady state during the last 4 hours of the 12 hour infusion. The apparent half-life following the infusion was 84 ± 3 minutes. Neither dose altered blood pressure, heart rate or ECG compared to vehicle control animals. Similarly, urinalysis and hematological results were within normal range for all groups. Cholesterol and triglycerides were modestly elevated; only the latter exceeded the normal range. Significant dose related increases of prothrombin time and activated partial thromboplastin time of 25% at the estimated clinical dose and 100% at five times that dose were measured at steady state. These values returned to baseline 24 hours post-infusion. Finally, no microscopic lesions or effects on bone marrow were associated with the administration of TFPI. Other than expected effects on coagulation, no significant toxicity was associated with TFPI at the exposures attained in this study.

556 ALTERED HEMOSTASIS IN MALE RATS TREATED WITH AN ACAT INHIBITOR

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Sponsor: D. Murphy

Male and female Sprague-Dawley rats were given oral doses (10, 100 or 400 mg/kg/day) of an Acyl-CoA:cholesterol AcylTransferase (ACAT) inhibitor for 6 months. Hemorrhage was observed in males given 400 mg/kg/day beginning at one month, prompting collection of blood samples at weeks 6, 17 and 24 to evaluate coagulation parameters. A dose-related increase in activated partial thromboplastin time (APTT) and Thrombotest™ TM clotting time (TCT, 1/51 method) was observed in all male drug-treated groups. Mean APTT values for male rats given 10, 100 or 400 mg/kg/day were increased to 17.5, 20.8 and 34.7 seconds (control: 15.4-16.0 seconds), and mean TCT values were increased to 86, 100 and >300 seconds (control: 71-74 seconds), respectively. Mean prothrombin times (PT) for male rats given 400 mg/kg/day were increased to 16.5 seconds (control: 12.9-13.1 seconds). Activity of factors II, VII, IX and X was decreased in males at dosages of 10, 100 or 400 mg/kg/day (73-89%, 61-87% and 13-21% of control activity, respectively). There was no consistent effect on factors V or VIII. We conclude that the pattern of change in coagulation parameters and its occurrence in males only are consistent with vitamin K deficiency and that the APTT and TCT are more sensitive than the PT in detecting deficiencies in vitamin K-dependent coagulation factors in this species.

557 TOXICITY STUDIES OF A MALARIA TRANSMISSION-BLOCKING VACCINE (TBV-25) IN RABBITS

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The toxicity of TBV-25, a 20 kD protein transmission-blocking antimalarial vaccine, was assessed in New Zealand White rabbits. Two *i.m.* treatment regimens using groups of 6 males were employed: 1) 0.5-ml at Days 0, 28, and 84 of either 50, 50, and 250 ug or 250, 250, and 50 ug of TBV-25 per injection, or 2) 1.0-ml at Days 0, 28, and 56 of 250 μg. Controls received the Alhydrogel alum vehicle in PBS, pH 7.0. No deaths or treatment-related clinical signs occurred. Slight, transient irritation at the injection sites occurred in both treated and control animals. Body weights, weight gains, rectal temperatures, and food consumption were comparable across all groups. Clinical chemistry, hematology, urinalysis, organ weights, organ-to-body weight ratios, and organ-to-brain weight ratios revealed no clinically significant effects. Organ weights and organ-to-body weight ratios for the liver and kidneys of treated rabbits in the second experiment were statistically lower than those of the control group (75.4 ± 12.3 g and 17.6 ± 1.6 g, respectively, vs 92.1 ± 10.8 g and 21.6 ± 1.6 g for controls). This difference appears to be due to higher than normal liver and kidney weights in the control rabbits in this experiment (historical control values were 69.6 ± 8.2 g and 17.8 ± 2.2 g). There were no microscopic findings related to treatment with TBV-25. Treatment with TBV-25 resulted in no adverse effects in these clinically relevant schedules. (Supported by NIAID Contract NIAID-DAIDS-NO1-AI-15111)

558 28-DAY INTRATHECAL INFUSION STUDY OF RECOMBINANT-METHIONYL HUMAN BRAIN-DERIVED NEUROTROPHIC FACTOR (r-metHuBDNF) IN DOGS

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R-metHuBDNF has been shown to have survival-promoting activity in animal models of motor neuron degeneration. Since motor neuron cell bodies in spinal cord are affected in amyotrophic lateral sclerosis (ALS), intrathecal administration is an attractive delivery route. Therefore the toxicity associated with continuous intrathecal infusion of r-metHuBDNF (200-2000μg/d) in dogs for 28 days was evaluated. There were no biologically significant effects noted in body weight, food consumption, blood chemistries, cerebrospinal fluid chemistries or organ weights. Significant findings from this study were reductions in hindlimb muscle tone and coordination. A dose-dependent increase in the incidence and severity of motor dysfunction was noted at 800-2000μg/day in the 2nd week of the study. Motor dysfunction was evidenced by muscular rigidity and stiffness, hindlimb discomfort, deficit in reflex responses, and circling. The time of onset and magnitude of the disorder were proportional to dose and were at least partially reversible during a two-week recovery. Histopathology of the spinal cords revealed no findings that could be attributed to r-metHuBDNF. This study demonstrates that continuous intrathecal infusion of 200μg/day r-metHuBDNF is well tolerated in dogs and does not appear to be associated with any significant effects on motor function.

559 ACUTE TOXICITY AND IRRITANCY OF 2,2-DIBROMO-2-NITROETHANOL (DBNE)

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DBNE (CASRN 69094-18-4), a biocide, was investigated for acute toxicity and irritancy. Undiluted DBNE of purity >98% was tested. *Acute Peroral Toxicity.* LD_{50} values (95% CL) in mg kg^{-1} were 62.7 (22.4-134) for male and 29.1 (0-44.8) for female Sprague-Dawley albino rats, and 107 (72-159) for male and 93 (60-154) for female Swiss Webster mice. Signs included hypoactivity, altered breathing pattern, salivation, and hematuria. Renal tubular degenerative lesions were seen. *Acute Percutaneous Toxicity.* LD_{50} values for New Zealand white rabbits (24-hr occlusion) were 354 (204-613) mg kg^{-1} for males and 297 (167-528) mg kg^{-1} for females. Signs included hypoactivity, altered breathing pattern, and hematuria. Acute renal tubular degenerative changes were present. *Acute Vapor Exposure.* Sprague Dawley albino rats exposed for 6-hr to a statically generated saturated vapor atmosphere produced neither mortality nor signs. *Skin Irritation.* 1 hr and 3 min occluded contact (0.5 ml) in New Zealand white rabbits produced moderate to severe erythema and edema; full-thickness necrosis was seen in all (6) animals by 1-hr contact and 1/6 with 3 min. *Eye Irritation.* 0.01 ml DBNE in New Zealand white rabbits produced severe conjunctival hyperemia, chemosis and discharge, and moderate corneal injury.

560 LOCAL AND SYSTEMIC EFFECTS FOLLOWING A SINGLE INTRA-ARTICULAR ADMINISTRATION OF RECOMBINANT HUMAN INTERLEUKIN-4 IN FEMALE MONKEYS

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Chronic inflammation noted in rheumatoid arthritis (RA) has been associated with the active production of cytokines by the synovium. IL-4 has been reported, in-vitro, to inhibit the spontaneous production of pro-inflammatory cytokines in RA synovial tissue. The local and systemic effects of intra-articular administration of rhuIL-4 into the knee were evaluated by conducting

a single administration study at doses of 0, 5 or 50 μg/kg in three female cynomolgus monkeys per dose level. Blood was collected between 0 and 24 hrs post-dose for IL-4 blood level evaluation. Following blood collection for clinical pathology determinations, necropsies were conducted three days post-dose. No rhuIL-4 related clinical signs, body weights, hematological or clinical chemistry changes were noted. At necropsy, minimal joint effusion was noted at the injection site in a few animals treated with 5 μg/kg. Histomorphologic changes were noted locally at the injection site and systemically in the cardiovascular system. Findings were slightly more pronounced at the 5 μg/kg dose level. At the injection site, findings consisted of inflammation of the synovium characterized by infiltration of a mixture of eosinophils, mononuclear cells and/or neutrophils. Cardiovascular system findings consisted of minimal or mild infiltration of scattered individual eosinophils in a few arteries and in the heart. Analysis of IL-4 serum level data indicated systemic exposure that was dose related but not dose proportional. The AUC and Cmax at 50 μg/kg (112,600 pg.hr/ml and 20,159 pg/ml, respectively) were approximately 20 to 25 fold greater than that noted at 5 μg/kg (5247 pg.hr/ml and 808 pg/ml, respectively). The Tmax was 1 hr for both dose levels. The inflammation in the knee and eosinophilic infiltrates in the cardiovascular system were considered to be the result of rhuIL-4 induced recruitment of inflammatory cells. These findings are consistent with the reported biological effects of IL-4 to stimulate synoviocyte IL-6 production and the chronic selective recruitment of eosinophils, basophils, T lymphocytes, NK cells and monocytes by augmenting their binding to endothelial cells through increased expression of VCAM-1 on endothelial cells and VLA-4 on leukocytes.

561 SPACIAL AND TEMPORAL DISTRIBUTION OF ULTRAVIOLET RADIATION IN PHOTOTOXICOLOGY STUDIES

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Several classes of pharmaceutical products have been identified with photodegradation, with photosensitized reactions or with other light/drug interactions. These products include antibiotics (such as fluorinated quinolones), non-steroidal anti-inflammatory drugs, and a variety of unrelated vasoactive compounds. Other compounds require "light" activation for their desired effect. Consequently, both safety and efficacy testing have increasingly depended on the availability of well-characterized sources of optical radiation and the means to document their spacial and temporal distribution of energy. This is particularly challenging for pre-clinical safety tests involving the simultaneous exposure of large surface areas (i.e., groups of animals or cages). We have constructed sources, using either xenon arc lamps or fluorescent lamps, capable of delivering uniform fluence rates to several square meters of target surface (i.e., with a standard deviation of a few percent of that at the center). The xenon arc solar simulator provides approximately 0.25 to 0.50 of a solar constant throughout the ultraviolet and visible spectral regions (i.e., from 290 to greater than 800 nm). The fluorescent lamps are particularly efficient in producing higher intensities in selected wavebands (e.g., 2 – 8 mW/cm2 of UVA; 340–380 nm), which is several times the intensity contained in earth-level sunlight. This presentation will provide examples of spacial energy distribution and algorithms for calculating such distributions.

562 SUBCHRONIC TOXICITY OF RECOMBINANT HUMAN INTERLEUKIN-10 (rhuIL-10) IN CYNOMOLGUS MONKEYS

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Interleukin 10 (IL-10) is an immunomodulatory cytokine that has been shown to inhibit antigen presentation by monocytes/macrophages *in vitro*, stimulate B-cell proliferation and function *in vitro*, and inhibit the synthesis of pro-inflammatory cytokines both *in vitro* and *in vivo*. The subchronic toxicity of *E.coli*-rhuIL-10 was evaluated by intravenous (IV) and subcutaneous (SC) dosing in cynomolgus monkeys at 5, 15 or 45 μg/kg (IV) and 1, 5, 15 or 50 μg/kg (SC) for one month; and 1, 5, 25 or 75 μg/kg (SC) for three months. The reversibility of rhuIL-10-induced changes was assessed over a one month recovery period. No antemortem findings were noted. Serum IL-10 concentrations were dose-related. Anti-rhuIL-10 antibody was detected at all doses and intervals (including recovery) with no significant neutralizing activity. In all studies, significant toxicologic changes were generally restricted to the high dose and were reversible upon cessation of dosing. Hematologic findings included a regenerative anemia (mild-moderate), transient thrombocytopenia (mild) and lymphocytosis (mild-occasionally marked). These changes appeared during weeks 1–2 and continued throughout SC dosing, but were only evident at week 4 by IV dosing. Necropsy findings included higher liver (IV only) and spleen (♀ only) weights. Histomorphologic changes included hepatic Kupffer cell hypertrophy and mononuclear cell infiltration, and a reactive splenic red pulp. Moderate amounts of hepatic and splenic iron were noted. Bone marrow cellularity was unaffected. These findings, together with a reticulocytosis (mild-moderate), no change in serum haptoglobin and no evidence of immune-complex-mediated red cell lysis, suggest extravascular hemolysis as a mechanism for the anemia. The No-Observed-Adverse-Effect-Levels (NOAEL) for one month administration were 15 μg/kg (IV) and 50 μg/kg (SC), and 25 μg/kg (SC) for three month administration.

563 TOLERABILITY OF INTRAVENOUS SODIUM CITRATE BUFFER IN VARIOUS ANIMAL SPECIES

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Sodium citrate is often used as a buffering agent in parenteral drug formulations. Anecdotal information indicates that large volumes of citrate buffer are toxic to animals. Therefore, a study was initiated to evaluate the maximum tolerated intravenous (bolus) dose volume of an isotonic solution of 20 mM sodium citrate in aqueous sodium chloride in mice, rats, rabbits, dogs and monkeys. Mice and rats were given dose volumes of 3, 10 or 20 mL/kg whereas rabbits, dogs and monkeys received increasing dose volumes based on tolerability. In mice and rats, doses of 20 mL/kg were associated with labored respiration, hypoactivity, ataxia, or body arching. A dose volume of 20 mL/kg administered to a dog was associated with prostration, respiratory distress and increased muscular tone. In monkeys and rabbits, a dose volume of 10 mL/kg was associated with labored or slowed respiration. A dose volume of 20 mL/kg administered to rabbits was associated with labored respiration, hypoactivity, hyperextension of limbs, body arching, or hypoactivity. In conclusion, a dose volume of 10 mL/kg of 20 mM sodium citrate administered as a rapid intravenous bolus injection was well tolerated in all animal species tested; larger dose volumes should be avoided.

564 SAFETY PHARMACOLOGY EVALUATION OF THE OXAZOLIDINONE, U-100766

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U-100766 is being evaluated for the treatment of a variety of infectious diseases. These studies assessed the safety profile of U-100766 after acute dosing in the cardiovascular, respiratory, gastrointestinal and nervous systems. Compared to a group of anesthetized dogs treated with equivalent volumes of vehicle, sequential i.v. infusion of U-100766 at 3, 10 and 30 mg/kg produced slight increases in cardiac index (15.5%), tidal volume (19.5%) and dynamic lung compliance (11.5%) at 30 mg/kg, and slight decreases in PR interval (4.2 and 8.1%) and respiratory rate (19.6 and 30.4%) and a slight increase in heart rate (5.3 and 12.8%) at 10 and 30 mg/kg, respectively. Oral dosing of 6.25, 62.5 and 100 mg/kg to conscious rats did not affect gastric secretion or intestinal fluid volume, but inhibited gastric emptying in males (79% and > 100%) and females (>100% and 83%) at doses of 62.5 and 100 mg/kg, respectively. $3x10^{-4}$ M, U-100766 had no effect on isolated guinea pig ileum tone or on the EC_{50} and maximum contractile responses to acetylcholine, histamine and $BaCl_2$. However, U-100766 showed a nonspecific inhibition of histamine- and $BaCl_2$-induced contraction. Oral dosing of 6.25, 62.5 and 100 mg/kg to conscious rats had no effect on indices of activity, sensorimotor and autonomic function and rectal temperature. The minor changes in cardiovascular and respiratory function elicited by U-100766 were not considered of toxicologic significance. The reduction in gastric emptying may explain the inappetence observed at the high dose of U-100766 used in the rat toxicology studies. These findings support the safety of U-100766 with respect to its effects on cardiovascular, respiratory, gastrointestinal and nervous systems.

565 CHRONIC TOXICITY, ONCOGENICITY AND MECHANISTIC STUDIES WITH PROPYLENE GLYCOL MONOMETHYL ETHER (PGME)

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The chronic toxicity/oncogenicity of PGME vapors was evaluated in B6C3F1 mice and F344 rats exposed for 6 hr/d, 5 d/wk for up to 2 yr at concentrations of 0, 300, 1000 or 3000 ppm. To characterize the metabolic and morphological bases for PGME-induced transient sedation and hepatic hypertrophy in mice and rats and kidney toxicity in male rats, satellite groups were exposed for 1, 2, 13, 26, 52 or 78 weeks and evaluated for enzyme induction and cell proliferation in the livers of mice and rats and cell proliferation and alpha2u-globulin in the kidneys of rats. PGME-induced sedation at 3000 ppm resolved in all animals during the first 1–2 wk of exposure as a result of adaptive changes in the liver (enzyme induction and cell proliferation). In rats only, enzyme activities dropped to near control levels by 52 wk coinciding with a return of sedation. In male rats, loss of adaptation was followed by a dose-related increase in altered hepatocellular foci by 2 yr of exposure to 1000 or 3000 ppm PGME. The kidney toxicity observed in male rats was confirmed as an alpha2u-globulin nephropathy. No statistically-identified increases in tumors were observed in either species; however, a numerical increase in kidney tumors (3/50) was observed in male rats from the intermediate exposure level. The overall chronic NOEL for rats was 300 ppm, discounting the alpha2u-globulin nephropathy, and 1000 ppm for mice. There were no treatment-related tumors relevant for human risk assessment.

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566 SUB-ACUTE TOXICITY STUDY OF MA-HUANG IN MICE

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The safety issue of a concentrated ma-huang (*Chinese Ephedra*) herbal preparation which contains 9% l-ephedrine was addressed in a sub-acute toxicity study in BALB/c mice. Five-week old male and female mice, 10 animals per dose (5 mice per sex), were given the herb orally by gavage at 4000, 2000, 1000, 200, and 20 mg/kg twice daily for two weeks. Two control groups were used, untreated and animals gavaged with water. Deaths and any signs of toxicity such as tremors, lethargy, hunching, ruffled fur, diarrhea, and prostration were noted daily, and as they occurred. The animals were sacrificed at the end of the study, and necropied to look for obvious organ abnormalities. The liver weights of the controls and the 2 high doses were also determined. The lethal dose for 50% of the test animals (LD50) was observed at the highest dose (4000 mg/kg), with gastrointestinal bleeding observed upon necropsies. The same toxicity findings, but to a lesser degree, were observed in the 2000 mg/kg groups; however, no significant differences were noted in the liver weights of these animals when compared to the control groups. The sex of the animals did not appear to be a factor among the groups exhibiting the forthmentioned signs of toxicity. The study indicated that mahuang is well tolerated in mice orally, regardless of sex, in doses as high as 2000 mg/kg twice daily. As stated before, the chief safety concern of ma-huang is its principle ingredient, l-ephedrine, which is 9% (wt/wt) in this ma-huang preparation. From these data, the LD50 of l-ephedrine in this herbal preparation is, therefore, determined to be 360 mg/kg twice daily in mice. When projected for human consumption of l-ephedrine and ma-huang based on surface area differences, the LD50 in a 70 kg human is calculated to be 2.1 and 23.3 grams twice daily, respectively; a quantity that greatly exceeds normal and traditional usages.

567 THE NEUROTOXICOLOGICAL ALTERATIONS INDUCED BY NARCOTIC DRUGS AND INDUSTRIAL CHEMICALS IN THE RAT ARE ASSOCIATED WITH QUANTITATIVE CHANGES IN GLIAL FIBRILLARY ACIDIC PROTEIN

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Diverse neurotoxic insults result in proliferation and hypertrophy of astrocytes, a subtype of glia in central nervous system. The hallmark of this response, often terms "reactive gliosis", is the enhanced expression of the major intermediate filament protein of astrocytes, glial fibrillary acidic protein (GFAP). These changes in the astrocytes suggest that GFAP may be a useful biochemical indicator of neurotoxicity. To investigate this possibility, we administered intra-peritoneally prototype neurotoxicants, methamphetamine (MAP, 5mg/kg), cocaine (30mg/kg), N-butyl benzenesulfon-amide (NBBS, 300mg/kg) and trimethyltin (TMT, 8mg/kg) to Wistar rats and then assessed the effects of these agents on the content of GFAP, which were determined by Sandwich ELISA and evaluated with neurotoxic symptoms, and quantitative changes of immunoreactivity of GFAP by light microscopic image analysis in specific regions. We found that assay of GFAP revealed time- and region-dependant patterns of neurotoxicity. The GFAP immunoreactivity of rat brain was increased in substantia nigra and hippocampus by MAP, NBBS and TMT; in medial septal nucleus and nucleus accumbens, was also increased by NBBS. Sandwich ELISA showed that GFAP levels of brainstem in all groups on 3 and 7 days and in MAP, NBBS groups on 1 and 3 days were increased. A review of the background, design and results of these experiments are presented in this paper. Our findings indicate that GFAP is a sensitive and specific biomarker of neurotoxicity.

568 RELATIONSHIP BETWEEN LEWIS^y ANTIGEN EXPRESSION AND TOXICITY FOLLOWING TREATMENT WITH THE MONOCLONAL ANTIBODY BR96

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BR96 is a human/mouse chimeric monoclonal antibody that binds to Lewis^y-related antigens (Le^y) that are expressed at high levels on various tumor types, but are also expressed on normal gastrointestinal (GI) epithelium. BR96 conjugated to doxorubicin (BR96-Dox) is being developed as an anti-cancer agent. Intravenous administration of BR96 or BR96-Dox results in acute gastrointestinal (GI) effects in some, but not all, beagle dogs. Flow cytometric and radio-immunohistochemical methods were developed to quantitate Le^y expression on single-cell suspensions and tissue sections, respectively. Initial studies, using single-cell suspensions obtained by colonic biopsy, demonstrated that Le^y expression in individual dogs correlated with signs of acute GI toxicity following treatment with either BR96 or BR96-Dox. Subsequent studies determined that flow cytometric analysis of Le^y expression on buccal epithelial cells, obtained by scraping the mouth of dogs, could also be used to predict acute GI toxicity. This screen has been utilized to select animals for prospective studies to assess the pharmacokinetics of BR96, and to evaluate the toxicity of other BR96-related compounds. In addition, analysis of Le^y expression on human buccal epithelial cells is being evaluated for its ability to predict acute GI toxicity in Phase I clinical trials.

569 PRE-CLINICAL SAFETY OF CTLA4Ig (BMS-188667): IMMUNOLOGIC FINDINGS

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CTLA4Ig (BMS-188667) is a novel recombinant DNA-derived protein that is being developed as an immunosuppressant. Preclinical safety studies were performed in mice and monkeys. No clinically significant drug-related changes were noted. No significant hematologic or immunologic changes were noted in single-dose monkey studies. In a repeat-dose monkey study (q2dx15), no hematologic effects were observed, but there was an apparent dose-related decrease in serum IgG levels. This effect was reversible, and was not clinically significant. CTLA4Ig was antigenic in both mice and monkeys; however, antibodies were not detected until after the completion of treatment. The anti-CTLA4Ig antibody response had a slow onset compared to a typical immunogenic protein, which suggests that anti-CTLA4Ig antibodies were not formed until after serum drug concentrations decreased to below immunosuppressive levels. As expected, mice treated with human CTLA4Ig produced significant anti-CTLA4Ig antibodies, and when these mice were challenged with CTLA4Ig they displayed clinical signs of hypersensitivity. Cynomolgus monkeys also produced anti-CTLA4Ig antibodies (although lower levels than in mice) and the presence of these antibodies correlated with a rapid decline of CTLA4Ig from serum. In conclusion, intravenous administration of CTLA4Ig was not associated with any clinically significant immunologic changes or any other drug-related toxicity.

570 THE TOXICITY OF ISIS 3521, A PHOSPHOROTHIOATE OLIGONUCLEOTIDE, FOLLOWING INTRAVENOUS (IV) AND SUBCUTANEOUS (SC) ADMINISTRATION IN CYNOMOLGUS MONKEYS

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ISIS 3521 is a 20-base phosphorothioate (P=S) oligonucleotide, antisense to human PKC-α, which is being developed for oncology applications. IV infusion of 20 mg/kg over 2 hr caused changes in mean arterial pressure and heart rate in one monkey, while 80 mg/kg SC had little effect on hemodynamics. The severity of hemodynamic changes tended to correlate with plasma C_{max} of ISIS 3521. While there was a high degree of variability in plasma ISIS 3521 concentrations following SC administration, alternative complement pathway activation (measured by Bb) was observed in both treatment groups. Consistent with other P=S oligos, repeated SC administration of 10 and 80 mg/kg (every other day for 4 doses) resulted in renal toxicity characterized by basophilic granularity of proximal tubular epithelial cells in both dose groups. Tubular degeneration was noted at 80 mg/kg only, and was associated with alterations in serum chemistry and urinalysis parameters. These toxicities were consistent with high concentrations of P=S oligo detected in tissues following a SC dose of 80 mg/kg. Significant complement activation was observed at 80 mg/kg SC, but only minimal activation was observed following 10 mg/kg SC. Additional alterations noted at 80 mg/kg included thrombocytopenia, Kupffer cell hypertrophy, hepatic inflammation, vacuolation of hepatocytes, and lymphoid hyperplasia in spleen and lymph nodes. These alterations have not been previously observed with other P=S oligos, and likely reflect higher tissue exposure as a function of dose.

571 MECHANISM OF SPECIES DIFFERENCES IN HEPATIC PORPHYRIA WITH A NOVEL ALKYL KETONE HERBICIDE

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2-[1-(Ethoxyimino)propyl]-3-hydroxy-5-(2, 4, 6-trimethylphenyl)cyclohex-2-enone (ETC) is a novel alkyl ketone herbicide. Administration of ETC to mice for 28 days resulted in severe cholestasis, due to accumulation of porphyrin in the liver which could be observed after a single dose. Rats and hamsters were insensitive to this hepatotoxicity. The sensitivity of mice to ETC-induced porphyria correlated with reduced liver ferrochelatase activity; this is the terminal enzyme of the haem biosynthetic pathway catalysing the incorporation of iron into protoporphyrin IX to form haem. Inhibition of ferrochelatase activity resulted from accumulation of N-methyl protoporphyrin IX in the liver, a potent inhibitor of this enzyme. The insensitivity of rats and hamsters to ETC-induced porphyria reflected a lack of accumulation of this inhibitor and hence lack of inhibition of ferrochelatase activity in the liver.

572 EVALUATION OF ACUTE TOXICITY, IRRITANCY AND DERMAL SENSITIZATION POTENTIAL OF TETRASILVER TETROXIDE (AG_4O_4)

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Silver compounds are widely used in numerous industrial applications, as human therapeutic agents and in potable water filtration systems. The studies reported here evaluated the safety of a new proposed water disinfectant that is formed *in situ* from precursor products that result in final concentrations of 1 ppm Ag_4O_4 and 10 ppm potassium persulfate activator. In acute toxicity/irritancy tests conducted at 4 fold the intended use concentration, the oral LD50 to Wistar rats was >5000 mg/kg bw. A 24-hr occluded dermal exposure of New Zealand albino rabbits (5/sex) to 2000 mg/kg bw produced transient, slight erythema and edema but no other signs of dermal toxicity. A 4-hr occluded exposure to 0.5 ml of the test material produced only slight erythema at 30–60 minutes that did not persist at 24 hr post-dosing. Instillation of 0.1 ml of the test material into the eyes of six rabbits produced slight, transient conjunctival redness and discharge that was absent by day 1. No dermal sensitization response was seen in guinea pigs using the Buehler method. In support of the safety of its proposed use in water disinfection, the test material exhibited a very low degree of oral, dermal and ocular toxicity and irritancy and lacked sensitization potential.

573 BROWN ADIPOSE TISSUE EFFECTS OF THE LTD_4 ANTAGONIST RG 12525 IN THE RAT: MECHANISTIC STUDIES

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RG 12525 is a potent leukotriene D_4 antagonist developed for the treatment of asthma in man. Chronic treatment of rodents with RG 12525 produced a marked dose-dependent increase in interscapular brown adipose tissue (BAT) weight and a significant increase in mitochondrial uncoupling protein (UCP). Histologically and ultrastructurally, the effects of RG 12525 on brown adipocyte tissue were characterized by cellular hypertrophy and hyperplasia, intracellular lipid changes ranging from micro to macro-vesiculation, nuclear eccentricity of brown adipocytes and an increase in connective tissue. In rodents, the activity of BAT is primarily regulated by the sympathetic nervous system (SNS). Stimulation of BAT by norepinephrine, cold exposure or β3-agonists produces significant effects on BAT, including increases in organ weight and induction of UCP. In this mechanistic study, the BAT effects produced by RG 12525 were investigated using BAT-sympathectomized and/or cold exposed rat models. Treatment of rats with RG 12525 before cold exposure resulted in better thermoregulation of core temperature (+0.6°C) as compared to the cold-exposed control group. This observation suggests that RG 12525 does not interfere with BAT functionality. The effect of RG 12525 on BAT weight was not found to be affected by sympathectomy, however, the induction of UCP, as well as the up-regulation of core temperature required intact sympathetic innervation of BAT. Unlike RG 12525, the increase in BAT weight with cold exposure required intact sympathetic innervation. These studies demonstrate that the pleiotropic effects of RG 12525 and cold exposure on BAT are not mediated by identical mechanisms nor is the BAT weight increase caused by RG 12525 a response to an inhibition of BAT functionality.

574 ECOTOXICITY HAZARD ASSESSMENT OF STYRENE

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The ecotoxicity of styrene was evaluated in acute toxicity studies of fathead minnows (*Pimephales promelas*), daphnids (*Daphnia magna*), amphipods (amphipods (*Hyalella azteca*), and freshwater green algae (*Selenastrum capricornutum*), and a subacute toxicity study of earthworms (*Eisenia foetida*). Stable exposure levels were maintained in the studies with fathead minnows, daphnids and amphipods using sealed, flow-through, proportional dilution systems. The algae were evaluated in a sealed, static system. The earthworms were exposed in artificial soil which was renewed after 7 days. Styrene concentrations in water and soil were analyzed using gas chromatography with flame ionization detection. Test results are based on measured concentrations. The LC50 (96 hr) for fathead minnows was 10 mg/l, the EC50 (48 hr) for daphnids was 4.7 mg/l, the LC50 (96 hr) for amphipods was 9.5 mg/l, the EC50 (96 hr) for green algae was 0.72 mg/l, and the LC50 (14 d) for earthworms was 120 mg/kg. Styrene's potential impact on aquatic and terrestrial environments is significantly mitigated by its volatility and biodegradability. Sponsored by the Styrene Information and Research Center.

575 RISK ASSESSMENT OF THE REPLACEMENT OF TRICHLOROETHYLENE BY TRICHLOROETHANE

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Trichloroethylene (TCE) has been the major industrial degreaser used in the U.S. until positive tests for carcinogenicity in laboratory rodents caused its demise. Today TCE is the most common toxicant found in Superfund sites across the U.S. On the basis of its alleged potential human carcinogenicity, the maximum contaminant level (MCL) for TCE in drinking water has been established at 5 μg/L. More than any other substance, TCE has been responsible for shutting down aquifers as drinking water sources. A similar volatile organohalide, 1,1,1-trichloroethane (TCA), has been used widely for the past two decades to replace TCE. Unlike TCE, TCA has not tested positive for carcinogenicity in rodent tests. However, the very long atmospheric half-life of TCA as compared to TCE make it a significant contributor to ozone depletion. Nine epidemiologic studies of extensively exposed TCE workers have proven negative for carcinogeneis. However, it is predicted that TCA

will contribute significantly to increased skin carcinogenesis through ozone depletion over the next half century.

576 CALTOX STOCHASTIC RISK ASSESSMENT COMPARED TO STANDARD CERCLA METHODS

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We have compared the results of evaluating selected soil contaminants (chloroform, DDT, toxaphene) at a hazardous waste site in California, using stochastic CalTOX methods and standard CERCLA methods. This site has soil contamination resulting from storage, formulation, and disposal of chemicals. CalTOX is a risk assessment model which relates contaminated soil to human health risk. CalTOX was run stochastically (Monte Carlo) and with CERCLA reasonable maximum exposure estimates to evaluate the contribution of the model structure itself. Depletion of the source of contamination by movement of chemicals among environmental compartments and degradation over time can have a considerable effect on risk assessment. It can decrease the estimation of risk from the soil while increasing risk in other compartments. For some chemicals, inclusion of food pathways using CalTOX makes a significant contribution to total risk estimation. Stochastic analysis provides information on the entire distribution of exposures for comparison to the point estimates which result from current deterministic risk assessment. The results of stochastic analysis will be useful to risk managers for making more informed decisions about remediation of contaminated sites.

577 EVALUATION OF THE ORAL AND INHALATION CANCER POTENCY FACTORS (CPFs) FOR ACRYLONITRILE (ACN)

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The current inhalation and oral CPFs for ACN were determined in 1983 by the USEPA (2.4 x 10^{-1} and 5.4 x 10^{-1} mg/kg/day, respectively) based on lung cancer rates in one epidemiology study and three chronic rat studies, respectively. Since 1983 a number of advances have been made in understanding the toxicokinetics, epidemiology, and potential mechanisms of ACN carcinogenesis. These new data were reviewed to ascertain whether they could be used to refine and update the existing CPF values. An update of the critical epidemiology study and studies of two additional cohorts did not find excesses of lung cancer. The calculations of the oral CPF, based on the chronic rat studies, assumed a genotoxic mechanism, that the "biologically active dose" was the applied dose, and that similar rates of metabolism occur in rats and humans. Recent data indicate that a non-genotoxic mechanism of ACN carcinogenicity is possible and demonstrates metabolic differences between rats and humans. Reevaluation of the CPFs for ACN is warranted at this time and should take into consideration the internal dosimetry of ACN and its metabolites in animals and humans as well as the impact of alternative mechanisms of carcinogenesis.

578 PREDICTIONS OF THE IMPACTS OF AIRBORNE LEAD ON CHILDREN IN CALIFORNIA AND THE INTER-RELATIONSHIP WITH EXPOSURES FROM OTHER MEDIA

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At low blood lead concentrations, lead may cause neurodevelopmental effects in children, especially one and two year olds. While a threshold level has not been identified, 10 μg/dL is regarded as the level of concern for children. Since epidemiologic studies use blood lead concentration as an exposure indicator, changes in air lead were related to concentrations of blood lead. A strong and consistent relationship of air to blood was found with a ratio of approximately 4.2 μg/dL per μg/m^3 for children. Data from NHANES III, representative of the U.S. population, were used to estimate blood lead distributions of one and two year olds in California, using a geometric mean of 4.1 μg/dL and a geometric standard deviation of 2.1. An "aggregate" model quantitatively related exposures from ambient air lead concentrations to the percent of additional children that might exceed the level of concern at increasing air lead concentrations. Results indicated that at an average ambient level of 0.06 μg/m^3 may elevate between 0.6% and 2.3% of the population of one and two year old children above 10 μg/dL. A 0.25 μg/m^3 hot spot may elevate 5–13% of the children. Most lead exposure may be attributed to other sources including tap water, paint, food or soil. It was estimated 10.9% of one and two year old children in California are predicted to have blood lead levels exceeding 10 μg/dL due to these other sources. Characteristics of the local environment may affect the prediction and interpretation of potential air lead impacts. The U.S.EPA IEUBK model was used to determine the inter-relationship of the various media and provide information on the most effective manner to reduce childhood blood lead levels.

579 A COMPARATIVE ANALYSIS OF A LINEAR METHOD FOR ESTIMATING CARCINOGENIC POTENCY WITH THE CONVENTIONAL LINEARIZED MULTISTAGE MODEL

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For cancer risk assessment, the linearized multistage model (LMS) has been widely applied to carcinogens for low dose extrapolation. This method has some limitations. For example, more attention needs to be paid to mode of action and exposure scenario. A new linear method is introduced for carcinogens which interact directly with DNA or for which the dose-response is expected to be linear at environmental levels. The new method calculates the slope of the line drawn between LED_{10} and the origin (zero dose, zero response). LED_{10} is defined as the estimated low dose corresponding to the upper 95% confidence limit at 10% response. LED_{10} is in contrast to ED_{10} which is based on the best estimate of dose at 10% response. In addition, a new inter-species scaling factor is applied (body weight raised to the 3/4 power instead of 2/3 power). The new linear method will be applied on ten carcinogens and the results will be compared to estimates obtained from the LMS model.

580 USE OF MONTE CARLO (MC) ANALYSES IN A MULTI-CHEMICAL, MULTI-PATHWAY HUMAN HEALTH RISK ASSESSMENT (HHRA) FOR A STATE SUPERFUND SITE (Part 1 of 4)

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This HHRA for a former solvent recycling facility was prepared to comply with a DTSC issued consent order. This is the first HHRA accepted by DTSC to incorporate probabilistic methods to estimate risk. The Site, containing polychlorinated biphenyls, chlorinated solvents and metals, is surrounded by residential and agricultural land. The planned future Site use is either residential or as a park. Due to the location of the Site, the public concern for the Site, and the need for effective risk communication, use of MC was agreed upon by ENVIRON and DTSC. The MC analyses-based HHRA provided more accurate site-specific risk estimates. Site conditions required evaluation of several exposure scenarios for which no default exposure parameters were available. The most scientifically defensible method to determine exposure parameters for these scenarios was to define ranges rather than choose upper-bound points. Pathway-specific exposure distributions were used to evaluate several possible exposure scenarios: on- and off-site residential; on-site park worker and visitor; and on-site hypothetical consumer of potable ground water. MC was used to estimate pathway-specific intake rate distributions. The 95th and 50th percentile intakes were multiplied by representative media concentrations to arrive at reasonable maximum exposures (RME) and average exposures, respectively. For some RME scenarios, MC risk estimates were 5 times less compared to point estimate risk calculations. Using MC methods lead to a more accurate prediction of risk for this Site and provided a tool for more effective risk communication.

581 USE OF CALTOX TO GUIDE SITE CHARACTERIZATION AND RISK MANAGEMENT DECISIONS FOR CONTAMINATED SOIL

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CalTOX is a multimedia, multiple pathway risk assessment model for relating contaminated soil to human health risk for specified exposure scenarios. The model is consistent with USEPA guidance on human intake and associated risk, but extends this guidance by adding an eight-compartment, source-depleting, transport model. The model is run on an EXCEL spreadsheet and is designed to be used stochastically. In this paper, risk estimates are compared

for a persistent, immobile chemical, benzo(a)pyrene (BaP), and for a mobile, labile chemical, trichloroethylene (TCE). Sensitivity analysis reveals that risk from potential TCE exposure is most affected by uncertainty associated with factors related to release and subsequent exposure to ground water. These factors include the soil degradation half-life and soil-water partition coefficient, Kd. In contrast, biotransfer factors for uptake into food had the greatest effect on uncertainty for exposure and risk due to BaP. By identifying specific parameters that most affect the results, CalTOX provides a rational basis 2for site characterization and suggests a broader array of risk management strategies which may be more cost-effective than those currently considered.

582 SITE-SPECIFIC EXPOSURE DISTRIBUTIONS FOR A STATE SUPERFUND HUMAN HEALTH RISK ASSESSMENT (HHRA) (Part 2 of 4)

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This HHRA incorporated Monte Carlo (MC) techniques to estimate distributions of human exposure to constituents present at a former solvent recycling facility. To conduct the MC analysis, each exposure assumption was defined as a probability distribution. In the last five years, more publications have provided information on ranges and distributions of various exposure parameters. In this HHRA, ENVIRON identified appropriate site-specific exposure distributions from data provided in key publications by USEPA, Cal/EPA, and other peer-reviewed literature. Due to the uniqueness of some site-specific exposure scenarios, professional judgement was required to define distributions for those exposures. ENVIRON worked closely with DTSC Cal/EPA as the lead regulatory agency, to select representative distributions appropriate for this Site. Since the DTSC has been developing suitable distributions for the CalTOX™ risk assessment model, those values were used when available and appropriate. Guidance on the distributional forms (e.g., lognormal, cumulative, triangular) was limited to a USEPA Region III memo and suggestions from DTSC. In the absence of regulatory guidance on the appropriate distributional forms, ENVIRON developed a hierarchy of the distribution types based on the quantity and quality of the available data. Data on exposure variability is an active area of research that is rapidly evolving. This project was an example of how MC risk assessment methods provide risk managers more accurate information about the types and magnitude of potential risks associated with exposure to a site. These exposure factor distributions were also used to develop health-based cleanup levels.

583 RISK ASSESSMENT OF METHYLMERCURY IN FISH

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Methylmercury (MeHg) bioconcentrates in aquatic food chains. To weigh the risks of MeHg exposure against the benefits of fish consumption, an risk analysis is presented of fetal and adult neurological impairment. It describes the magnitude of the harm, anticipated variability and uncertainty of the estimates expressed as a range of alternative outcomes. The analysis includes a probabilistic description of MeHg exposure from the consumption of fish, including tuna, shark and swordfish. Uncertainty is associated with the use of short-term consumption surveys (e.g. underrepresention of low-frequency consumers, exaggerate high-end exposures). A clinical study of the chronic diet to blood relationship (Sherlock et al., 1984) was used to model population variance. The relationship between blood and hair mercury (Hg) levels was evaluated using a survey of women of childbearing age (Marsh et al., 1985). The hair Hg to adverse effects relationship of the fetus and adult was based on the study of Marsh et al. (1987) of the Iraqi poisoning episode. MeHg intake was simulated for 2000 individuals using different control levels for MeHg in fish. Each simulation consisted of 600 uncertainty iterations with each iteration representing a different interpretation of the data. Each uncertainty iteration consisted of 2000 variability iterations, each one representing an individual in the population. Results are presented as percentiles and risk estimates calculated from the net increment attributable to the change in MeHg exposure. An increase of about 1% above background levels of CNS dysfunction is attributed to MeHg in fish and placement of various control levels reduces the added risk from 10 to 40%.

584 DEVELOPMENT OF A TOXICOKINETIC MODEL FOR PREDICTING ADVERSE EFFECTS OCCURRING FROM INHALATION EXPOSURE TO CADMIUM

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Cadmium renal and respiratory tract toxicity in humans was modeled based in part on Oberdorster (*Principals of Route-to-Route Extrapolation for Risk Assessment*, 1990, p.217) to provide a quantitative answer as to which tissue is more sensitive to continuous air exposure to Cd. Based on default breathing rates, fractional pulmonary deposition derived from the model used by U.S. EPA and the fractional solubility of cadmium oxide, systemic absorption from pulmonary deposition of Cd was estimated at 3.78 μg per 1μg Cd/m^3 per day. For pulmonary effects, the 95% lower bound on urinary excretion level associated with a 10% increase in the probability of adversely low forced ventilatory capacity was 23.4 μg Cd/l urine excreted/day (Smith et al., Am. Rev. Resp. Dis. 14:161, 1976). This level of urinary Cd excretion was associated with a continuous air exposure of 8.7 μg Cd/m^3. For renal effects, 200 μg Cd/g within the renal cortex was adopted as a critical concentration (Kjellstrom et al., Env. Res. 33:284, 1984) with a $t_{1/2}$ of 20 years. A continuous air exposure to 1.9 μg Cd/m^3 was estimated for maintenance of the kidney at the critical concentration indicating kidney effects would be expected to be manifest before the pulmonary effect specified above.

585 ORAL CARCINOGEN POTENCY FACTORS FOR BENZENE AND SOME OF ITS METABOLITES

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After absorption, benzene (BZ) is metabolized to a large number of intermediates, three of which are hydroquinone (HQ), catechol (CC), and quinone (QN). BZ has been linked to nonneoplastic and neoplastic hematopoietic effects, particularly the myelogenous cell types, in humans exposed by inhalation. Rodents exposed to BZ develop neoplasms at numerous sites including Zymbal's gland, Harderian gland, preputial gland, oral cavity, lungs, hematopoietic system, skin, mammary gland, and ovary. Rodents exposed to HQ develop neoplasms in the kidney, hematopoietic system, and liver; whereas neoplasms develop primarily in the glandular stomach of rodents exposed to CC, and in the hematopoietic system of rodents exposed orally to QN. Oral carcinogenicity data were available for dose-response assessment of BZ and the three metabolites. These data were evaluated for adequacy to derive carcinogen potency values The carcinogen potency factors were calculated as 1/ED10s, derived from the dose corresponding to a 10% tumor incidence or risk (ED10); the data were not extrapolated below the experimental range. The ED10s were derived using the linearized multistage model. Carcinogen potencies for HQ and CC are very low <0.22 or 0.07 (mg/kg/day)$^{-1}$, respectively. The estimated potency of 11 (mg/kg/day)$^{-1}$ for QN is 50-fold higher than that of HQ. The estimated carcinogen potency for BZ is quite low, <1.0 (mg/kg/day)$^{-1}$. These data suggest that oral exposure to BZ is associated with a low hazard to humans because the average intake of BZ from drinking water is 0.2 μg/day.

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586 USE OF PHARMACOKINETIC MODELING IN CANCER RISK ASSESSMENTS FOR REGULATORY PURPOSES

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Physiologically based pharmacokinetic (PBPK) models are increasingly used in cancer risk assessment for dose-response extrapolation between species or routes of exposure and from high to low doses. Some generic problems limit their usefulness in regulatory applications. These include: the weight of evidence required to characterize reactive intermediates, the extent of data in humans needed for reliable interspecies extrapolation, and difficulties in assessing the pharmacokinetic contribution to site specificity in different species. Unresolved technical issues include the appropriate level of model complexity, the characterization of uncertainty in model parameters, levels of model validation, and the description of heterogeneity in human pharmacokinetic processes. These issues are explored in the following risk assessment projects:

1. The cancer potency of vinyl bromide (VBR) was estimated (Salmon *et*

al., Toxicologist 12:96, 1992) based on liver angiosarcoma incidence in female Sprague-Dawley rats. A PBPK model for VBR in the rat was developed, using published experimental data. Evidence for human heterogeneity in relevant enzyme systems was examined.

2. Cancer risks from exposure to chloroform in chlorinated swimming pool scenarios were estimated with a human PBPK model similar to that described by Corley *et al. (Toxicol Appl Pharmacol* 103:51, 1990). The effect of varying physiological parameters on predicted metabolism of chloroform in liver and kidneys was investigated using allometric relationships and a Monte Carlo method.

The discussion reviews the treatment of key pharmacokinetic issues, including critical decision-points and explicit acknowledgement of uncertainty, and provides recommendations for further development of PBPK models for regulatory use.

587 CANCER RISK ASSESSMENT FOR CHLOROFORM

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Chloroform is a common chlorination by-product in drinking Water. EPA has regulated chloroform as a probable human carcinogen under the Safe Drinking Water Act. The cancer risk estimate via ingestion was based on the 1985 Jorgenson study identifying kidney tumors in male Osborne Mendel rats. This assessment researched the correlation between certain direct (e.g. necrosis) and indirect (e.g. cell proliferation) parameters of cytotoxicity and tumorigenicity reported by available studies. Variables such as species, sex, strain, target tissue, dose level, dose vehicle, and exposure duration are specified. There is a good correlation between certain cytotoxic effects of chloroform and the occurrence of liver tumor in female B6C3F1 mice (via DW and corn oil gavage). It appears that there is little or no correlation between certain cytotoxic effects and tumorigenicity in: 1) liver of male Fischer-344 rats (DW), 2) liver & kidney of Fischer-344 male rats (inhalation), 3) kidney of male B6C3F1 mice (corn oil gavage), and 4) kidney of male Osborne-Mendel rat (DW and corn oil gavage). Cancer risk in the kidney was compared using the data from male rats administered with chloroform by corn oil gavage (NCI, 1976) or by drinking water (Jorgenson et al. 1985) using LMS model. Because of concern over the possible effects of unequal survival rates in animals in different dose groups, the data for these studies were also modeled using time-to-tumor method. There are no significant differences in kidney cancer risks using data of rats exposed to chloroform by corn oil gavage or by drinking water.

588 HUMAN HEALTH ASSESSMENT OF FRESHWATER FISH CONTAMINATED WITH MERCURY

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Bioaccumulation of organic and inorganic mercury in freshwater fish in the Gulf-South region of the United States has resulted in public health authorities issuing fish consumption health advisories and other public health warnings. This risk assessment research examines the current toxicological literature and exposure assumptions to develop models for assessing potential public health impact of fish consumption, and the role of fish interspecies variability in assessing the potential for bioaccumulation. Our risk assessment models indicate that a continuous diet of fish with 1.0 mg Hg/kg in the edible portion, which is the current Food and Drug Administration (FDA) action level, is inappropriate for pregnant and breast-feeding women and children less than seven years of age. A more appropriate action level for this sensitive subpopulation would be 0.5 mg Hg/kg in the edible portion of fish. Toxicological risk assessment models were applied to a 100 mile stretch of the Ouachita River in northeast Louisiana beginning at the Arkansas line where mercury levels in some bass exceeded the FDA action level. The risk assessment models for mercury resulted in an approach for providing separate fish consumption information for sensitive subpopulations and the general public. The general models developed may have application in other fish contamination situations where the primary health endpoint is systemic toxicity and not cancer risk.

589 COMPARISON OF RISK ESTIMATES DERIVED FROM STANDARDIZED POINT ESTIMATE, TRADITIONAL MONTE CARLO ANALYSIS, AND MICROEXPOSURE ANALYSIS FOR THE DOMESTIC USE OF TRICHLOROETHYLENE IMPACTED GROUNDWATER

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A risk assessment was performed to determine the health effects from domestic use of groundwater impacted with trichloroethylene (TCE). The selected routes of exposure were ingestion, dermal contact from bathing, and inhalation of volatilized TCE. The doses and associated risks were calculated over 30 years using three approaches: (1) standardized point estimate; (2) traditional Monte Carlo Analysis (MCA); and (3) microexposure MCA. All three approaches used the same TCE concentrations, which varied over time and location along the centerline of the plume. For the point estimate approach, USEPA exposure assessment parameters were used as the default values in calculating the risks. For both of the MCAs, 5,000 persons were randomly selected along the plume to calculate the distribution of risks among the population. The traditional MCA used USEPA distributions for exposure parameters to calculate risks. For the microexposure MCA, the individuals were assigned starting ages and percentiles for the various exposure parameters. Each year, the exposure parameters were selected from distributions based on age and percentile. The cumulative lifetime risk was tracked until the person either moved or died. The risks per million at the 50th and 90th percentiles were: 4 and 11 for the point estimate, 0.6 and 4 for the traditional MCA, and 0.2 and 1 for the microexposure MCA. Both of the MCA approaches had lower risks due mostly to lower inhalation rates, showering times, exposure durations, and ingestion rates. The microexposure MCA had lower risks than the traditional MCA due to the high mobility of the actual population.

590 METHYL-*TERT* BUTYL ETHER (MTBE) MODE OF ACTION: IMPLICATIONS FOR DOSE-RESPONSE

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MTBE's tumorigenicity has been evaluated in three bioassays, which differ in the species and strain of laboratory animal tested and route of exposure. The inhalation bioassays show statistically significant increases in kidney and testitular tumors in male rats and livers of female mice; a statistically significant decrease in endometrial hyperplasia was observed in mice. Male rat kidney tumors were observed in association with chronic progressive nephrotoxicity. Similarly, female mice demonstrated signs of liver and systemic toxicity. When administered via olive oil gavage to Sprague-Dawley rats, MTBE caused an apparent incidence of combined leukemias and lymphomas in female rats, and an increase in Leydig cell tumors in males. Because MTBE is not genotoxic, these tumor responses may be associated with two potentially overlapping modes of action: 1) Toxicity and compensatory proliferation at the target organs (e.g., male rat kidney tumors, female mouse liver tumors), or 2) Gender-specific hormonal modulation of tumorigenesis (e.g., female mouse liver and decreased endometrial hyperplasia). With either of these modes of action, the dose-response for MTBE-induced tumors may exhibit a toxicity-associated thresholds. Biologically-based models of dose-response for carcinogenic risk assessment must consider pharmacokinetics and the relative contribution to toxicity made by MTBE and its metabolites. MTBE is oxidatively metabolized to tertiary butyl alcohol (TBA) and formaldehyde (FA). The amount of FA formed following MTBE administration does not exceed amounts formed during normal metabolism. Although TBA is not genotoxic, chronic administration in drinking water produced an increase in male rat kidney tumors. It is thus possible that the male rat kidney tumor increases observed in the inhalation study may be caused either by MTBE or TBA. A critical issue for low-dose risk assessment is thus determination of the ultimate toxicant(s) and the point(s) at which MTBE and TBA metabolism become(s) saturated.

591 USE OF STOCHASTIC MODELS TO CHARACTERIZE VARIATION IN LIFETIME, CHRONIC, AND SUBCHRONIC DOSE RATES IN AN EXPOSED POPULATION WITH TEMPORAL AND AGE-RELATED VARIABILITY

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Characterizing the distribution of dose rates received by a population exposed

to an environmental contaminant is a critical portion of the risk assessment process. Researchers have used stochastic models to characterize variation in long-term dose rates in exposed populations such as the lifetime average daily dose. In this analysis we present the result of a microexposure event model that characterizes the distributions of dose rates for different time scales. The result of the model is a characterization of the distribution of lifetime, chronic, and maximum subchronic doses rates for an exposed population. The model achieves this by modeling each individual's exposures as a series of separate exposure events. The dose rates for each event are modeled separately and summed over different periods of time to provide estimates of the different dose rates. The results of the analysis demonstrate that, in certain cases, the distribution of maximum annual dose rates can have a higher mean and variation than the distribution of lifetime average daily doses. The results of this model can be used to evaluate the distribution of risks for chemicals or mixtures of chemicals that pose both chronic and subchronic hazards. A major advantage of the approach is that it provides a consistent means of characterizing different types of dose rates.

592 COMPARISON OF METHODOLOGIES FOR ESTIMATING DIETARY EXPOSURE TO ABAMECTIN RESIDUES IN FOODS

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Most risk assessments significantly overestimate potential risks from dietary exposure to pesticides. Meat and milk are large components of the U.S. diet so the methodology used to estimate intake of residues from these foods is particularly important. Additional contributors to exposure estimates are 1) assumptions concerning levels of residues in samples without quantifiable residues and 2) the potential time-frame of exposure. A case study was performed for the pesticide abamectin to identify 1) the relative magnitude of overestimation and 2) data and methods to improve the assessment without the need for additional studies. Data generated from controlled field trial studies were used to estimate potential residues in foods and livestock feeds. To estimate residue levels in meat and milk, information about commercial animal feeding practices were utilized in conjunction with U.S. EPA guidelines. Food consumption patterns were delineated using data for approximately 15,000 subjects collected by USDA from 1989–1992. Joint probability distribution of exposure using Monte Carlo simulations, were computed. Exposures were matched to the appropriate toxicological endpoints. Under the most conservative assumptions, the exposure estimate for the upper 95th percentile of the U.S. population was 0.00055 mg/abamectin/kg BW/day; whereas under a more realistic estimate, the exposure was 0.00004 mg/abamectin/kg BW/ day. These values were computed for a single days' intake and are, therefore, comparable to toxicology studies in which exposure was for a short period of time.

593 HEALTH-BASED CLEANUP LEVELS (HBCLs) USING MONTE CARLO (MC) ANALYSIS OF EXPOSURE DISTRIBUTIONS (Part 3 of 4)

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HBCLs are estimated concentrations of chemicals in an environmental medium that are based on: a human population's predicted contact rate with the media; the cancer and noncancer toxicity criteria; and a regulatory approved target cancer risk and noncancer hazard index. Typically, HBCLs use upper-bound point estimates for each exposure parameter because these are the variables in the HBCL calculation with the greatest uncertainty. Combining these upper-bound exposure parameters in the HBCL calculation often leads to overly conservative values. Also, point-estimate HBCLs provide little flexibility for risk managers because uncertainties associated with upper-bound values are not available for consideration. This project uses MC analysis for distributions of human exposure parameters to calculatemulti-pathway HBCLs for a California State Superfund Site. The 50th and 95th percentile distributions of HBCLs were developed for receptors of two potential post remediation land uses: residents; and maintenance workers and visitors to a public park. This use of MC analysis of exposure parameters to derive HBCLs has three particular advantages for risk management decisions: 1) uncertainties (sensitivity analysis) inherent in the exposure parameters can be considered; 2) distributions of HBCLs for multiple populations allows the FS to focus on cleanup alternatives for exposures of greatest concern for a particular site use; and 3) exposure parameters driving key exposure pathways for each population and land use are highlighted. This paper discusses the procedures for using MC to set HBCLs, and exposure parameters driving the key exposure pathways for Site populations and land uses.

594 RISK MANAGEMENT DECISIONS USING MONTE CARLO RISK ASSESSMENT RESULTS (Part 4 of 4)

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The objective of using a Monte Carlo (MC) risk evaluation is to quantify uncertainties associated with risks for a site and enhance opportunities to make informed risk management decisions. This California State Superfund project also developed a range of health-based cleanup levels (HBCLs) from the MC exposure distributions. State statute SB923 encourages evaluation of potential land uses to include residential as well as others. Accordingly, two future land uses were considered: residential, and a public park. The results provided risk managers with a range of possible cleanup levels to reduce risks. Key constituents of potential concern are chlorinated solvents, PCBs and metals. HBCLs as well as Maximum Contaminant Levels and Ambient Water Quality Criteria (if available) were evaluated for relevant media as potential cleanup goals. Selection of implementable cleanup goals must consider technical feasibility along with issues such as: exposure assumptions driving estimated risks and their uncertainties; the severity of toxicological endpoints controlling risks; the population densities of those being protected; cost differential associated for the risk reduction range; post remediation land uses; and community concerns. Interim measures at this site previously removed source area soils. A ground water treatment system is currently operating to contain plume movement. This paper discusses cleanup decisions regarding: remaining soils; ground water; off-site soils; and potential downgradient ground water release to surface water in a public park and a large lake.

595 DEVELOPMENT OF A STOCHASTIC LEAD EXPOSURE MODEL FOR USE WITH THE U.S. EPA IEUBK MODEL FOR LEAD IN CHILDREN

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An integrated stochastic exposure (ISE) model was developed for quantifying human exposure to lead via multiple exposure pathways (e.g., soil, dust, paint chips, tap water, diet, and inhalation). Using MicroExposure Monte Carlo modeling techniques, variability and uncertainty (expressed as probability distributions) were assigned to exposure variables to characterize age-specific physiology and activity patterns, demographic and housing conditions, and environmental concentrations of lead. The ISE model was linked to the biokinetic module of the U.S. EPA Integrated Exposure, Uptake, and Biokinetic Model (IEUBK) for Lead in Children. The linked model was used to conduct an uncertainty analysis of childhood lead poisoning among an urban population at the census tract level. Probability distributions for blood lead concentrations were evaluated with respect to a wide range of plausible exposure scenarios for children ages 0.5- to 7-years old. A sensitivity analysis identified key exposure variables, including ingestion rate, percent lead in paint, and the concentration of lead in soil. Results of hypothetical simulations demonstrate how the efficacy of lead abatement programs in terms of reduced blood lead concentrations varies among the exposed population. Furthermore, the model is used to assess the importance of modeling assumptions, including time steps used to simulate temporal variability in parameter values, and correlations among key exposure variables. The methodology has applications to risk assessments of other compounds as well as older age groups that may be susceptible to the health effects associated with lead exposures.

596 A PHARMACODYNAMIC MODEL OF ENDOCRINE MODULATOR EFFECTS ON THE SPRAGUE-DAWLEY (S-D) RAT ESTROUS CYCLE

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Cessation of ovulation in Sprague-Dawley rats begins at about 12 months of age, leading eventually to an anovulatory state, characterized first by persistent estrus (PE), prolonged exposure to endogenous estradiol (E2) and polycystic ovaries. This first phase of reproductive senescence is due to the inability of the hypothalamus (HYPO) to support an effective GnRH-mediated, ovulation-inducing, lutenizing hormone (LH) surge, rather than ovarian

exhaustion. Several compounds, including chlordecone (CD) and the s-chlorothiazine herbicide atrazine (ATRA), first induce lengthened cycles and then PE. We developed a pharmacodynamic estrous cycle (PD-EC) model for the S-D rat that focuses primarily on interactions between LH and E2. E2 has positive and negative feedback effects on LH release from the pituitary but produces hypothalamic toxicity. E2 also mediates transcription in the HYPO leading to synaptic remodeling. Our model assumes that failure of the LH surge and ovulation ensue when cumulative-E2 toxicity leads to insufficient HYPO-E2 receptor reserve to accomplish remodeling in the intercycle period. The model was calibrated by examining data on altered cycle characteristics and PE induced by ATRA. The most intriguing model-derived insight was the prediction that both weak functional agonists (i.e., CD) and weak functional antagonists (i.e., the triazine herbicides) could lead to early onset PE. The behavior occurs because of interactions between organs with little receptor reserve, e.g., HYPO, and organs with spare receptors, the ovaries, uterus, breasts, etc. Weak functional agonists and antagonists preferentially affect E2-responses in receptor poor tissues (the LH surge mechanism in the HYPO) without overt effects on receptor-rich organs. This PD-EC model may be useful in evaluating toxic endpoints caused by various endocrine modulators, such as xenobiotic-induced mammary tumors in S-D rats, and for determining whether threshold doses are likely to be associated with those responses. (This work was supported by CIBA-Crop Protection).

597 DEVELOPING PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PB-PK) MODELS FOR MANGANESE AND IRON

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Iron (Fe) and manganese (Mn) are essential transition series elements which exist in the body in the +2 and +3 valences states. Fe transport throughout the body is regulated by a carrier protein, transferrin (Tr), and tissue storage of that portion of Fe not involved in protein function or O_2 storage occurs in a ferritin (Ferr), a specific Fe-binding protein, and hemosiderin. To a large extent, Mn disposition follows the Fe pathways. Despite tight control, both metals are toxic in overload conditions. We have developed PB-PK models of Fe and Mn transport to aid in interpreting nutritional and toxicological studies with these elements. The model includes Mn kinetics for different valence states and for inhalation, ingestion, and intraperitoneal and intravenous dosing routes. An Fe model was also developed to form the basis of considering interactions between Fe status and Mn disposition. In the model, Mn^{+2} is oxidized in the blood and tissues to Mn^{+3} and is also eliminated in the bile. This fecal elimination of Mn^{+2} is the only loss route. The model was used to successfully describe time-course data in the literature from rats, monkeys, and humans exposed to Mn^{+2}, M^{+3}, and mixed valence compounds. Deep tissue stores of Mn, presumably essential Mn in enzymes, do not equilibrate rapidly with newly absorbed Mn leading to biphastic disposition kinetics. In the homeostatic region, intestinal uptake, and blood uptake from intestinal cells are closely adjusted to elimination. In overload, the excess intake intake associated with both saturable and first-order uptake processes is not eliminated sufficiently rapidly, resulting in accumulation of free or loosely bound Mn in target tissues and in toxicity. One suggestion from this preliminary model is that Mn overload may result from less avid binding of excess Mn to Ferr compared to Ferr binding of Fe. With refinement, these models should be useful in developing non-cancer exposure guidelines for Mn which acknowledges its essentiality.

598 BIOLOGICALLY-BASED RISK ASSESSMENT MODELS FOR DIOXIN HEPATOCARCINOGENISIS SUPPORT NON-LINEAR LOW DOSE EXTRAPOLATIONS

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Studies with dioxin and other liver tumor promoters support a negative selection mechanism of tumor promotion. First, dioxin acts as a weak mitogen; the body then adapts by elaborating mito-inhibitory growth factors; finally, specific liver cells, unresponsive to mito-inhibition, grow out to clones of altered cells. We linked physiologically-based pharmacokinetic (PB-PK), gene induction, and clonal growth models to describe the dioxin tumor promotion does-response curve. The PB-PK/gene induction portion accounts for (1) dioxin binding to the Ah receptor and to cytochrome P4501A2, both of which are induced by dioxin, and (2) induction of cytochromes and alterations of cell growth parameters in the liver by Ah receptor-dioxin-DNA complexes in a regional and dose-dependent manner. The gene induction model provides input to the clone growth model where 2 populations of intitiated cells respond differentially. Growth of one population is suppressed by the mito-inhibitory environment; growth of the second, unaffected by the anti-proliferative milieu, is enhanced at higher, hepatotoxic doses of dioxin. In our model, mito-suppression is correlated with centrilobular induction of proteins; clonal expansion of resistant cells is correlated with the panlobular induction at higher doses. We used the model to analyze liver tumor promotion/ protein induction data from sub-chronic studies from 1 to 125 ng dioxin/ kg/day. The simulated dose-response curves are U-shaped with a region where dioxin actually reduces the growth of altered cells compared to controls. Based on this highly-nonlinear behavior, the risk assessment for dioxin hepatocarcinogenisis should be based on a margin-of-exposure factor (100 to 1000) and corrections for differences in kinetics between animals and humans. Linear extrapolations do not appear warranted for this endpoint. (Supported in part by the American Forest and Paper Association).

599 ANGLER-CAUGHT FISH CONSUMPTION DISTRIBUTIONS FOR ANGLERS AND THE GENERAL PUBLIC FOR IMPROVED PROBABILISTIC RISK ASSESSMENT

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Probabilistic risk assessment requires distributions of various inputs to characterize their inherent variability. Consumption of angler caught fish can be a significant exposure pathway particularly for lipophillic toxicants. The Santa Monica Bay Fish Consumption Study (1994) is a well conducted survey addressing a diverse California angler population. SAS® was used to derive distributions from the raw data from this study. Systat® was used to develop the associated probability plots. The fish consumption distribution derived from this study reveals fish consumption is highly skewed indicating a lognormal distribution. The mean, standard deviation, skewness, and kurtosis are 49.6 g/day, 81.7 g/day, 5.83, and 50.0, respectively. This distribution indicates that anglers can be sensitive subpopulation because of their high consumption rates relative to the general population. In order to determine risk to the general population from angler-caught fish consumption a second distribution was derived from the angler fish consumption. The number of combined fresh and salt water fishing licenses was determined and divided by the population over 16 (licensed anglers/population). The fraction of anglers (0.06) was multiplied times the average California household of 2.7 to yield the fraction (0.16) of the California population who consumed angler-caught fish. This fraction was multiplied times the angler fish consumption distribution to yield an angler-caught fish consumption distribution for the general public. The mean, standard deviation, skewness, and kurtosis are 8.73 g/day, 19.6 g/day, 9.59, and 129.3, respectively. These distributions allow a more accurate determination of the risk to anglers and the general population from consumption of angler-caught fish. These risks have been underestimated using standard point estimates of fish consumption.

600 ASSESSMENT OF AIRBORNE HEXAVALENT CHROMIUM IN THE HOME FOLLOWING USE OF CONTAMINATED TAPWATER

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Field studies were conducted to estimate the plausible uptake of hexavalent chromium [Cr(VI)] aerosols inhaled during indoor residential use of a shower and an evaporative cooler supplied with water containing Cr(VI). In the evaporative cooler study, water concentrations of 20 mg Cr(VI)/L did not produce an increased concentration of airborne Cr(VI) inside the home. In the shower study, the average airborne concentrations of Cr(VI) aerosols at breathing zone height ranged from 87 to 324 ng Cr(VI)/m^3 when the water concentration of Cr(VI) was 0.89 to 11.5 mg/L. The Cr(VI) concentration in air was directly correlated to water concentration. The lifetime average daily doses and incremental cancer risk estimates corresponding to 30-year residential exposures were calculated using the measurements in this study and published exposure guidelines. The plausible upperbound lifetime cancer risk associated with continuous exposure to "background" Cr(VI) in outdoor air was estimated at 6.9 per million for a person exposed from age 0-30, and 4.0 per million for age 30-60. Similarly, estimated upperbound cancer risks due to inhaling shower aerosols (10 minutes per day, every day) from water containing 2-10 mg Cr(VI)/L over the same exposure period ranged

from 0.9 to 5.5 per million. Our calculations demonstrate that shower aerosols do not contribute appreciably to background Cr(VI) exposures and risks at water concentrations up to 10 mg/L.

601 CHRONIC REPRODUCTIVE EFFECTS ARE SEEN IN DAPHNIA *MAGNA* EXPOSED TO A NON-STEROIDAL AROMATASE INHIBITOR, CGS 16949

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D. magna exhibits multiple P450 enzyme activities which can hydroxylate exogenous testosterone (Baldwin and LeBlanc, 1994). The capacity to produce estrogens via aromatase, an ancient cytochrome P450 which converts androgens to estrogens, has not been demonstrated. My preliminary results indicated that aromatase activity is present in adult *D. magna*. Subsequently, I performed a 28-day chronic *D. magna* reproduction assay to assess the effects of exposure to CGS 16949, a non-steroidal aromatase inhibitor used in human breast cancer treatment. A concentration of 24 μM CGS (LC_{50} = 250 μM) delayed the onset of reproduction by 1–2 days in the first generation and caused a significant decrease (one-way ANOVA, $p = 0.05$) in the number of neonates produced. A further 28-day chronic assay using neonates from the second brood with continued exposure to the same concentrations of CGS 16949 indicated that, while total numbers of neonates produced by treated animals was not different from controls, exposure to 24 μM CGS caused a marked phase change in the reproductive cycle. No significant difference in total protein measured per milligram dry weight was seen in either the first or second generation. These results suggest a role for estrogens in the reproductive cycle, but the mechanisms of action and consequences are unknown.

602 EVIDENCE OF GLUTATHIONE S-TRANSFERASE π IN HUMAN UTERINE TISSUE

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The glutathione S-transferase (GST) π-isoform is a cytosolic enzyme, principally involved in the detoxification of reactive metabolites. It is well established that the risks of either cervical or endometrial cancers are linked to cigarette smoking. No reports exist on the localization of GST isoforms in human cervical or endometrial tissues. We report here immunohistochemical evidence for the localization of GSTπ in these regions. We have studied uterine tissue obtained from normal and endometrial adenocarcinoma patients. Sections containing cervix (n = 12), endometrium (n = 11) and adjacent tumor regions (n = 5) were prepared from paraffin embedded, archival tissue blocks fixed in formalin. Tissues were stained by the avidin-biotin complex method with monoclonal antibodies against placental GSTπ. Semi-quantitative staining intensity was determined by microscopic examination of the tissues. Significant staining intensity was observed in the superficial squamous epithelial cells in 100% of the normal cervix which decreased in magnitude towards minimal staining in the basal cells. This pattern was not different in the cervix from the endometrial cancer patients, however predominantly nuclear staining was observed in the cervix from the cancer patients. This is the first report of immunohistochemical evidence of GSTπ in the squamous region of human cervix. Furthermore there seems to be a transition from the cytosolic to nuclear regions in this cell type for cancer patients. The staining intensity in the endometrial glands was minimal in both the normal and cancer patients. However significantly increased staining was observed in the tumor regions, indicating increased expression of GSTπ in tumor tissues.

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603 PREPUBERTAL INSENSITIVITY TO CARBENDAZIM-INDUCED TESTICULAR TOXICITY

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Previously we have shown that the benomyl (BNL) metabolite carbendazim (CBZ) and not BNL itself is the most likely mediator of BNL-induced testicular toxicity. CBZ is also a potent inhibitor of testicular microtubule assembly and this action is thought to underlie testicular toxicity. Literature reports have indicated that prepubertal animals lack sensitivity to testicular damage after BNL. The present study has utilized CBZ to investigate hypotheses which could explain prepubertal insensitivity to BNL/CBZ. In *in vivo* studies, CBZ (100 mg/kg, ip) was administered to postpubertal (90–110 days) and to prepubertal (30–35 days) rats. Twenty-four hours later, testes were collected and prepared for histopathological evaluations. CBZ caused little testicular damage in prepubertal rats but in adult rats, CBZ treatment resulted in sloughing of the seminiferous epithelium. When the inhibitory effect of CBZ on prepubertal testicular microtubule assembly was compared to postpubertal, the IC50 was actually lower in the prepubertal than postpubertal animals. By isoelectric focusing, no apparent differences in the pattern of b-tubulin isotypes was observed. Pharmacokinetic studies revealed the most likely reason for the differential sensitivity of young vs. adult animals. High levels of CBZ were found in the adult testes yet none were detected in testes from young animals 1 hr and 2 hr after administration of CBZ (164 mg/kg, ip). These data suggest the age-dependent differences in susceptibility to toxicity are due to pharmacokinetic differences and do not reflect a differential responsiveness of testicular cells. Supported by NIH grant #ESO5707.

604 THE HOBSON TRACKER AS A SUCCESSFUL TOOL IN REGULATORY TOXICITY STUDIES FOR THE EVALUATION OF RAT FERTILITY

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The Hobson Tracker enables continuous tracking of motile sperm over an unlimited period of time. The system has been validated for use in a GLP environment and has been shown to be useful in the assessment of fertility when a known anti-fertility agent is used (α - chlorohydrin). In particular changes in VSL have appeared to be of interest. Consequently the system has been used as a tool in a number of regulatory reproductive studies where fertility is being assessed in the conventional manner. In 5 of the 6 studies which have been evaluated, the fertility levels as measured by number of pregnant animals, corpora lutea, conception rates and live embryos were normal, as was the Hobson Tracker data. However, in the 6th there was a significant reduction in both VSL and semen concentration in the high dose group. When the fertility data was assessed (independently) there was a noticeable reduction in conception rates and live embryos. These results lend support to the argument that CASA is of value as a tool for predicting reductions in fertility levels. An enlarged data pool may produce sufficient evidence of the alteration in sperm motility parameters for a reduction/cessation of rodent fertility studies and their replacement with CASA.

605 EFFECT OF SUBCHRONIC ORAL MTBE EXPOSURE ON REPRODUCTIVE ENDOCRINE FUNCTION AND METABOLISM IN MALE RATS

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Methyl-t-butyl ether (MTBE), a widely-used motor fuel oxygenate, caused Leydig cell tumors in chronic studies of rats exposed to 8000 ppm by inhalation or 1000 mg/kg by gavage. MTBE also produced renal tumors in male rats and liver tumors in female mice, and induces P-450 in rats and mice. To explore the possibility that high doses of MTBE cause tumors as a result of an imbalance of reproductive hormone levels, male Sprague-Dawley rats were gavaged with MTBE in corn oil for one month. MTBE doses were initially 1500 and 1000 mg/kg, and were lowered to 1000 and 750 mg/kg to maintain body weight. Serum testosterone decreased in the higher MTBE dose group within hours after the first treatment ($p<0.05$), but was no different from controls at the time of sacrifice. Liver total P-450 was slightly elevated in the higher dose group after one month of treatment ($p<0.05$). These effects were not observed at the 750 mg/kg dose. Liver and androgen-dependent organ weights were unchanged. An immediate, transient decrease in serum testosterone is more consistent with a direct effect on the hypothalamic-pituitary-gonadal axis or stress of treatment. Biologically significant long-term changes in steroid metabolism might occur if P-450 induction is sustained.

606 MONO-(2-ETHYLHEXYL) PHTHALATE RAPIDLY ALTERS TESTICULAR GERM CELL APOPTOSIS

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Phthalic acid esters, used as plasticizers in food packaging and medical devices, reduce fertility and induce testicular atrophy in laboratory animals. The Sertoli cell of the seminiferous epithelium is recognized as the primary cellular target for phthalate-induced testicular injury. Phthalate-induced alterations in Sertoli cells lead to a rapid disruption of the Sertoli-germ cell physical

interaction resulting in germ cell loss from the seminiferous epithelium. To further characterize the consequences of phthalate-induced Sertoli cell toxicity, we investigated the the influence of mono-(2-ethylhexyl) phthalate (MEHP), on germ cell apoptosis in young (28 d.o.) rats. The incidence of germ cell apoptosis was measured in testis cross sections by nucleotide 3' end-labeling of fragmented DNA. In young rat testis there is normally a significant incidence of germ cell apoptosis that occurs to limit the clonal expansion of immature germ cells. Treatment of rats with MEHP (2 g/kg, p.o.) resulted in an early (3 h) and significant inhibition (30%) in the incidence of germ cell apoptosis. However, by 12 h, a significant increase (38%) in germ cell apoptosis was observed. To explain these results, we propose a model of Sertoli cell-directed germ cell apoptosis analogous to the mehanism by which cytotoxic T lymphocytes kill infected target cells. This mechansm involves both the Fas receptor and its ligand (FasL). Using RT-PCR, we have identified the presence of mRNAs for Fas and FasL in the testis, with a marked induction in their expression after MEHP exposure. These results suggest that MEHP alters the regulation of Fas-mediated germ cell apoptosis in the testis.

607 REPRODUCTIVE SCREEN OF MODULAR ARTILLERY CHARGE SYSTEM ADMINISTERED IN THE DIET OF SPRAGUE-DAWLEY RATS

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An artillery propellant under development by the U.S. Army is a granular mixture of 98% nitrocellulose, nitroglycerin, nitroguanidine, and <2% cryolite and graphite. The propellant, Modular Artillery Charge System, or MACS, consists of a single increment of propellant charge contained within a rigid combustible casing. As part of the process to develop environmental and health effects criteria, a 90-day modified Screening Information Data Set reproductive assay was performed. Male and female Sprague-Dawley rats were treated with diet containing either 0.0, 0.2, 1.0, or 2.0 g propellant/kg diet. No mortalities occurred and body weights were unaffected by treatment. Methemoglobin concentrations of the high-dose rats measured at 28 days and at the conclusion of the study were significantly elevated (23 to 25%) compared to control rats. Relative organ weights of treated animals did not differ from weights of their respective control groups. No adverse effects occurred in mating or fertility indices. No significant treatment-related differences were noted in length of gestation, sex ratio, gestation index, or mean number of pups per litter. (Supported by Department of Defense Contract No. F33615-90-C-0532)

608 LACK OF TRANSGENERATIONAL REPRODUCTIVE EFFECTS FOLLOWING TREATMENT WITH DI-ISONONYL, PHTHALATE (DINP)

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DINP (CAS RN 68515-48-0) was administered in diet to four groups of parental (P1 and their adult offspring P2) male and female Sprague-Dawley rats at concentrations of 0, 0.2, 0.4, and 0.8 percent. There were no treatment-related differences in clinical observations, survival, reproductive organ weights or histopathology, male mating, male and female fertility, female fecundity, or female gestational indices in either generation. Some evidence of reduced body weight gain in the high dose P1 females was seen during the postpartum period, which correlated with reduced food consumption. There were no significant differences in survival, clinical or gross observations for offspring in either filial generation (F1 or F2). Small, but statistically significant lower mean offspring body weights for F1 and F2 groups were seen, but were within the historical control range of the laboratory and not considered biologically important. The P2 generation successfully produced normal, healthy offspring after exposure to DINP in utero, during lactation, puberty, and adulthood, which indicates its lack of transgenerational effects on reproduction. In conclusion, DINP did not produce evidence of reproductive or fertility effects under the conditions of this study.

609 REPRODUCTIVE EFFECTS OF THIOPHENOL IN S-D RATS ASSESSED BY THE CONTINUOUS BREEDING PROTOCOL

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The potential reproductive toxicity of thiophenol in S-D rats was evaluated using the Reproductive Assessment by Continuous Breeding protocol. Thiophenol was nominated for testing due to the relative lack of reproductive toxicity information; it is used as a chemical intermediate and mosquito larvicide. Thiophenol in corn oil was administered by oral gavage at doses of 9, 18, and 35 mg/kg/day to 20 animals/sex/group. Rats were individually housed for one week and then cohabitated for 16 weeks. During cohabitation, litters were euthanized after evaluation on postnatal day (PND) 1. Litters born after Week 17 were reared until PND 21 and selected weanlings were administered the same dose levels as their respective parents. On PND 81 ± 10, F_1 animals were cohabitated within groups for one week and necropsied following delivery of the litters. During 16 weeks of cohabitation, adjusted live pup weight was decreased by 4 and 6% in the 9 and 35 mg/kg dose groups, respectively. At the crossover mating trial to determine the affected sex, the pups born to 35 mg/kg females weighed 8–9% less; no effects were observed in litters from thiophenol-treated males. In the F_1 mating trial, live F_2 pup weight was decreased by 9 and 12% in the 18 and 35 mg/kg dose groups, respectively. Throughout the study, the body weights of the F_0 and F_1 35 mg/kg males were consistently decreased. F_0 and F_1 liver and kidney weights increased with increasing dose and were associated with centrilobular hepatocellular hypertrophy and renal tubule degeneration. Decreased (5–6%) sperm motility was observed in the 18 and 35 mg/kg F_0 group. Inhibited spermiation was observed in all treated F_1 males but not the F_0 males. In conclusion, thiophenol is not a selective reproductive toxicant, because the effects on male (decreased sperm motility and inhibited spermiation) and female (decreased live pup weight during the crossover mating) endpoints occurred concomitant with, or at doses greater than, those that produced hepatic or renal effects.

610 MUCOSAL IRRITATION AND FERTILITY STUDIES IN THE RABBIT WITH *n*-DOCOSANOL 10% AND 12% CREAMS

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n-Docosanol is a broad spectrum antiviral compound that is currently in Phase III clinical trials for the treatment of recurrent herpes labialis. It has also been demonstrated to protect rhesus monkeys against vaginal challenges with SIV and is being considered as a prophylactic treatment against sexual transmission of HIV in humans. Since *n*-docosanol would be applied topically to the vaginal and penile tissues, vaginal and penile mucosal irritation studies were conducted in New Zealand White (NZW) rabbits. A fertility study was also conducted in NZW rabbits since *n*-docosanol would be used by women of child-bearing age. In the mucosal irritation studies, 40 female and 40 male rabbits were divided into groups of 10 animals and treated intravaginally (vaginal irritation study) or topically (penile irritation study) for 10 consecutive days with either 0.9% sodium chloride, *n*-docosanol 10% cream, *n*-docosanol 12% cream, or a 1:1 mixture of *n*-docosanol 12% cream and Gynol II with Nonoxynol-9. An additional group of 10 females was similarly treated with Gynol II with Nonoxynol-9 to serve as a comparative control. Following the last treatment, the vaginal and penile tissues were removed and processed for histopathological evaluation. In the fertility study, 32 NZW females in groups of 16 animals received an intravaginal dose of *n*-docosanol 12% cream or K-Y® Jelly prior to mating. The treated females were mated with breeder males, and their fertility evaluated on gestation day 10. The penile mucosal irritation study indicated that neither of the *n*-docosanol creams produced notable mucosal irritation and both were comparable to the 0.9% sodium chloride and Gynol II with Nonoxynol-9 groups. For the vaginal irritation study, mucosal irritation scores were well within the acceptable range of this study for all materials evaluated. In the fertility study, the copulation index and fertility of the females appeared unaffected by the *n*-docosanol treatment.

611 1, 3, 5-TRINITROBENZENE TOXICITY IN BOVINE TESTICULAR TISSUE-SLICES: TWO-DIMENSIONAL ELECTROPHORETIC ANALYSIS

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Advances in tissue-slice technology and a recent novel application of this technique to reproductive toxicology using bovine testis have demonstrated the remarkable utility of this approach. The object of the present study was to combine this *in vitro* toxicity screening system with large-scale two-

dimensional polyacrylamide gel electrophoresis (2DE) to detect and study alterations in testicular-slice protein patterns as molecular correlates of 1,3,5-trinitrobenzene (TNB) toxicity. Previous studies have shown that testicular slices remain viable for >24 hr and, as measured by protein synthesis inhibition, TNB causes dose-related injury. Tissue slices were prepared from bovine testicles, incubated for 2, 4 or 6 hours using Teflon/viton/titanium rotors and exposed to either 100μM, 500μM or 1mM TNB in the incubation medium. Slices were collected, solubilized for 2DE, and separated by large-scale ISO-DALT® electrophoresis. Resulting 2D protein patterns were then examined by image analysis. Statistical analysis of the protein patterns revealed variation coefficients comparable to patterns obtained from fresh rodent organ samples. Furthermore, specific protein alterations exhibited dose related inductions and declines in protein abundance, protein additions and deletions, as well as alterations in specific protein charge. Many of the alterations were progressive over time. Testicular slices exposed to the DMSO vehicle alone exhibited distinct protein pattern alterations. The results of this investigation demonstrate the toxicologic utility of combining *in vitro* tissue-slice technology with high-resolution 2DE protein mapping. This consolidation of methods offers a novel approach for the study of male reproductive toxicology, reduces experimental cost, and replaces the need for lab animals. (Supported by AFOSR Grant #F49620-93-1-0297).

612 AN ANIMAL MODEL FOR ASSESSING EFFECTS OF TOXICANTS ON SPERM NUCLEAR ACTIVATION

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Few male reproductive toxicology tests evaluate effect(s) of toxicants on sperm function. Endpoints of toxicity commonly measured (sperm count, morphology, motility) convey little information about the mechanism of toxicity (except motility), nor predict fertility or assisted reproduction success. We have developed an assay of a critical sperm function, the capacity of its nucleus to be activated at fertilization. Sperm nuclear activation is a set of biochemical events necessary for successful fertilization that occur upon the sperm's entry into the egg. These events, mediated by egg cytoplasmic factors, include chromatin decondensation, DNA replication, and chromatin recondensation. We have developed a rat sperm activation assay (RSAA) to study the effects of reproductive toxicants on sperm nuclear activation. The RSAA was developed by modifying a human fertility assay, the human sperm activation assay (HSAA; Brown et al., Fertil Steril 64:612-22, 1995). Our sperm activation assays involve incubating permeabilized sperm in ^{3}H-TTP-spiked*Xenopus laevis* frog egg extract. Factors in the extract activate the sperm nucleus inducing the biochemical events described above. Decondensation and DNA synthesis were assessed using an Image Analysis system and autoradiography, respectively. We have recently shown that human sperm chemically damaged *in vitro* by a reversible crosslinking agent respond abnormally in the HSAA (Sawyer et al., Reprod Toxicol 9:351-7, 1995). We now plan to expose rats to male reproductive system toxicants and assess any resulting functional alterations of the sperm nuclei.

613 ANTISPERM AUTOANTIBODIES (ASA) IN SERA OF MEN OCCUPATIONALLY-EXPOSED TO HEAVY METALS

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Previously, we have shown that exposure to lead (Pb) or mercury (Hg) results in autoantibodies to nervous system (NS) proteins, suggesting NS damage. The male reproductive system is another site targeted by these metals, with ensuing damage. Both NS and the reproductive system have limited immune activity. To test whether autoantibody detection could be used to evaluate damage to the reproductive system, an ELISA to detect serum ASA (IgA, IgM, and IgG) was developed and field-tested. Men exposed to Hg at a fluorescent light factory (n=39), Pb at a battery factory (n=50) and a reference group (R) of workers at a food packing plant (n=39) were studied. 17 women in the same industries were used as negative controls, and serum from 3 men who had undergone vasectomy acted as a positive standard. Sera from the women and R were negative for ASA. ASA were detected in groups exposed either Pb or Hg. Immunoglobulin isotypes IgM and IgA levels were significantly higher in Hg-exposure compared to Pb-exposure. ASA levels correlated significantly with years of metal exposure ($r=0.5$; $p=0.0001$), blood Pb ($r=0.3$; $p=0.05$) or urinary Hg ($r=0.5$; $p=0.0001$). The present study suggests that ASA detection may be used to indicate environmental damage to the male reproductive system. This and earlier studies of the NS suggest that the presence of autoantibodies may indicate a compromise in the 'immunoprivileged' status provided by blood-NS or blood-testes barriers. (Sponsored by NIH Center Grant ES-00260).

614 A COMPARATIVE ASSESSMENT OF FLOW CYTOMETRIC PLOIDY RATIOS AND HISTOPATHOLOGIC EVALUATION: EFFECTS OF 2-METHOXYETHANOL ON RAT SPERMATOGENESIS

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The purpose of this preliminary study was to investigate the possible augmentation of standard qualitative histopathological evaluation of testicular tissues with quantitative analysis using flow cytometric analysis of testicular preparations. Sprague Dawley rats (approximately 8 weeks old) received dosages of 0 (vehicle), 100, 250 or 500 mg/kg/day of the testicular toxin, 2-methoxyethanol, for 10 consecutive days by oral gavage. Twenty-four hours after the last treatment one testis was fixed, stained and evaluated histologically. The other testis was used to collect fresh spermatogenic cells and to determine relative ploidy ratios of propidium iodide stained spermatogenic cells demonstrating hypohaploid (elongating spermatids), haploid (round spermatids), diploid (spermatogonia and spermatocytes) and tetraploid (spermatogonia and spermatocytes undergoing mitosis) DNA using flow cytometric analyses. Histopathologic findings were consistent with the published literature on 2-methoxyethanol which identifies the primary spermatocytes undergoing pachytene development as the primary target. Decreases in subsequent stage secondary spermatocytes and step 1 round spermatids were observed in 100 mg/kg/day treated rats. Earlier leptotene/zygotene spermatocytes and later stage round spermatids also showed degenerative changes following multiple dosage levels of 250 and 500 mg/kg/day. Flow cytometric analysis revealed a dosage-related alteration in ploidy ratios at all dosage levels. A significant hypohaploid increase accompanied a haploid peak decrease indicating a depletion of round spermatids. In rats receiving 500 mg/kg/day, a diploid peak increase was shown to reflect an increase in somatic (non-germ) cells being analyzed due to a total spermatogenic cell decrease. This initial study indicates that determination of ploidy ratios by flow cytometry may be a useful quantitative tool to assist in the histopathological evaluation of spermatogenesis.

615 SERTOLI CELLS ARE THE LIKELY TARGETS OF MOLINATE TOXICITY

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Exposure of male laboratory animals to molinate via the oral route, or via inhalation caused a decrease in fertility, abnormal sperm morphology, decreased epididymal sperm numbers, and/or testicular degeneration. Decreased fertility, as evidenced by significant ($P<0.05$) pre-implantation loss, was also observed in female rats, mice and rabbits mated with males of the same species which had been exposed to molinate by gavage. Neither serum LH, nor serum androgen levels were affected by exposure to molinate through the oral, or inhalation routes. Therefore, molinate does not exert its antifertility effects through disruption of the gonadal-hypophysial hormonal axis. This tends to rule out the Leydig cells as a target of molinate. If the epididymis were the target of molinate, the effects would have been observed almost immediately. Yet, antifertility effects in male rats were not observed until the third week following exposure. The 3-week latency in observed effects suggested that molinate affected the later stages of spermatogenesis. The process of spermatogenesis in rats requires approximately 56 days. Yet, at 63 days post-dosing, damaged spermatocytes and spermatids were still apparent in the rat testes. As molinate and metabolites are rapidly cleared from the body (75% of a molinate dose was excreted within 24 hours), it is unlikely the effects were due to residual molinate. Spermatogonia damaged by molinate would have already completed the process of spermatogenesis by 63 days. In the testes, the only other cell type intimately involved in spermatogenesis is the Sertoli cell. Therefore, the Sertoli cells are probably the targets of molinate.

616 FERTILITY STUDIES IN MALE AND FEMALE RATS WITH THE HMG-CoA REDUCTASE INHIBITOR, ATORVASTATIN

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Atorvastatin is a highly efficacious HMG-CoA reductase inhibitor which produces greater reductions in LDL-cholesterol in hyperlipidemic patients than other lipid-regulating drugs. In a male fertility study, rats received 20, 100, or 175 mg/kg atorvastatin for 11 weeks prior to mating with untreated females, throughout mating, and until sacrifice. Body weight gain suppression (17% and 25%), and food consumption suppression, occurred in males at 100 and 175 mg/kg during the premating treatment period. There were no effects on copulation, fertility, # days to mating, reproductive organ weights, sperm counts, motility or morphology, and there were no effects on numbers of implants or live fetuses. In a female fertility study, rats received 20, 100, or 225 mg/kg for 2 weeks prior to mating with untreated males, throughout mating, and until Gestation Day (GD) 7. Body weight gain suppression (35%) occurred at 225 mg/kg during G D 0–8. There were no effects on estrous cycles, copulation, fertility, number of days to mating, viable litters, corpora lutea, implants, live fetuses or pre- and postimplantation loss. These studies demonstrate no adverse effects of atorvastatin on fertility and reproduction in rats.

617 DOSE (AND TIME DEPENDENT) BLOCKADE OF PREGNANCY IN SPRAGUE-DAWLEY RATS ADMINISTERED AMMONIUM DINITRAMIDE IN THE DRINKING WATER

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Ammonium dinitramide (ADN) is a class 1.1 explosive oxidizer, which may be used in solid rocket propellant mixtures and explosives. A 90-day general toxicity/reproductive screen performed on this compound at concentrations of 162, 103, 29, and 0.0 mg ADN/kg/day resulted in a treatment-related adverse effect on litter production. Numbers of animals producing litters (1/11, 3/12, 12/12, and 11/12) and numbers of pups per litter (means of 7, 7, 16, and 15) were significantly less than controls. In a follow-up study, mated dams treated with ADN at the same does concentrations as the previous study and examined at Gestation Days 10 and 20 showed a similar effect in fetus loss as those in the reproductive screen. A pre- versus postimplantation dosing regimen indicated that implantation is vulnerable to ADN effects during the preimplantation period (Gestation Days 1–3). No implantation sites were found in the rats treated with 2 g ADN/L drinking water during Gestation Days 1–3. Numbers of implantation sites found in the rats treated in the same manner during Gestations Days 4-8 were similar to those found in the control group. The pituitary was not specifically identified in this study as the site of primary action, but serum progesterone was reduced by 27%, which may have resulted in reduced fertility. (Supported by Department of Defense Contract No. F33615-90-C-0532)

618 EFFECTS OF SIX-MONTH DAILY SUBCUTANEOUS TREATMENT WITH ATOSIBAN, AN OXYTOCIN RECEPTOR ANTAGONIST, IN FEMALE RATS

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Daily subcutaneous administration of 2, 6 or 20 mg/kg/day of atosiban, an oxytocin antagonist, for 6 months was evaluated in female CD rats. Dose-related irritation at the injection sites was observed at 6 and 20 mg/kg/day. It was characterized grossly by eschar, ulceration, and scab formation and microscopically by fibroplasia, inflammation, and necrosis. Slight decreases in total protein, albumin, and cholesterol values at 6 and 20 mg/kg/day and mild decreases in calcium, and increases in phosphorus and fibrinogen concentration at 20 mg/kg/day were considered to be drug-related. Mild to moderate decreases in urine volume were observed at all dose levels and were accompanied by increased urinary protein and urine specific gravity at 6 and 20 mg/kg/day. This mild antidiuretic activity was not unexpected since some analogues of oxytocin exhibit antidiuretic activity. Decreased uterine and pituitary weights and increased ovarian weights occurred at 6 and 20 mg/kg/day. There were no microscopic changes in the pituitary or uterus. However, the ovaries had increased numbers of corpora lutea suggesting that the luteal life span may be prolonged. This is likely to be a pharmacologic effect since oxytocin shortens luteal life span in some species. Thymic atrophy, increased adrenal weight, and decreased total protein and albumin observed at 6 and/or 20 mg/kg/day were thought to be related to chronic stress resulting from irritation at the injection site. Plasma concentrations showed that atosiban was rapidly absorbed and eliminated, with apparent linear pharmacokinetics; and with no drug accumulation over time. Other than effects thought to be related to irritation at the injection site, serum biochemical, organ weight changes, and microscopic findings appeared pharmacologic in nature.

619 REPRODUCTIVE EFFECTS OF CAFFEINE IN S-D RATS ASSESSED BY THE CONTINUOUS BREEDING PROTOCOL

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Caffeine, the most widely consumed drug in the US, was tested using the Reproductive Assessment by Continuous Breeding protocol to determine if the delay to conception observed in humans consuming caffeine could be duplicated in S-D rats. Caffeine was administered via oral gavage at doses of 12.5, 25, and 50 mg/kg/day to 20 animals/sex/group. Rats were individually housed for one week and then cohabitated for 16 weeks. During cohabitation, litters were euthanized after evaluation on postnatal day (PND) 1. Litters born after Week 17 were reared until PND 21 and selected weanlings were administered the same dose levels as their respective parents. On PND 81 ± 10, F_1 animals were cohabitated within groups for one week and necropsied following delivery of the litters. During 16 weeks of cohabitation, live F_1 pup weight adjusted for litter size was decreased by 7, 7, and 8% in the 12.5, 25, and 50 mg/kg dose groups, respectively. The crossover mating trial revealed no differences in male or female reproductive parameters. A decreased number of live F_2 pups per litter (21%) and proportion of pups born alive (4%) was observed in the 50 mg/kg dose group. Throughout the study, the body weights of the F_0 and F_1 12.5 (6–12%), 25 (11–18%), and 50 (15–23%) mg/kg animals were less than controls. No treatment-related gross or microscopic lesions were observed in the F_0 or F_1 animals. Computer-assisted sperm analysis revealed treatment-related decreases in sperm motility, velocity, and average radius in both generations. The delay to conception observed in humans consuming caffeine was not observed in this study. Thus, caffeine is not a selective reproductive toxicant, because the minor effects on sperm motion parameters and reduced pup weights, litter size, and pup survival, occurred concomitant with, or at doses greater than, those doses that produce reductions in body weight.

620 SHORT TERM REPRODUCTIVE AND DEVELOPMENTAL EFFECTS OF SODIUM BROMATE IN S-D RATS WHEN ADMINISTERED IN THE DRINKING WATER

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$NaBrO_3$ was studied to identify possible toxicities of water disinfectant byproducts. S-D rats were evaluated in a modified short-term reproductive and developmental toxicity protocol (Harris *et al.*, 1992). $NaBrO_3$ was administered in the drinking water at dose levels of 25, 80, and 250 ppm over a 35-day period to one group of male rats (10/group) and two groups of female rats, Group A (10/group to test for effects during conception and early gestation) and B (13/group to test for effects during late gestation and birth). These concentrations produced estimated daily doses of 2.6, 9.1, and 26.4 mg/kg/d. Rats received dosed water at the following intervals: males - Study Day (SD) 6–34/35, Group A females - SD 1–34, Group B females - Gestation Day 6 - PND 1 (birth). Five days prior to exposure, males were cohabitated with the Group B females. Females were allowed to litter, observed through PND 5 and a uterine exam performed. Endpoints included maternal body weight, food and water consumption, number and weight of pups, and number of uterine implantations. Group A females were cohabitated with the males for up to five days (SD 13–18) and on SD 34, the number of corpora lutea, live and dead fetuses, and implantation sites were recorded. After 29/30 days of dosing, males endpoints included clinical pathology evaluations, organ weights, sperm analysis, and histopathology. There were no changes in the reproductive data for either female group or for male and female body weights, food and water consumption, clinical observations, and gross findings. Although male organ weights, clinical pathology, and histopathology were comparable among dose groups; there was an 18% decrease in epididymal sperm density at 250 ppm. Therefore, under this exposure regimen, $NaBrO_3$ may be a selective male reproductive toxicant at 250 ppm since male reproduc-

tive endpoints were altered. Future studies should more fully evaluate this effect over a greater exposure duration.

621 FLUTAMIDE-INDUCED RAT TESTICULAR TUMORS AND LYPOXYGENASE ACTIVITIES ARE REDUCED BY CALORIC RESTRICTION

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Leydig cell hyperplasia and adenomas are common in several strains of rats, including the Fischer 344. Numerous drugs and chemicals increase in the incidence of hyperplasia and adenomas when tested in rodent bioassays, while rodents on a calorically restricted diet have a significantly lower incidence of these pathologies. Luteinizing hormone-dependent release of arachidonic acid is a major regulatory step in Leydig cell steroidogenesis and prostaglandin synthesis. We tested the hypothesis that caloric restriction inhibits spontaneous cell proliferation and tumorigenesis through altered arachidonic acid metabolism and that caloric restriction would decrease the incidence of flutamide-induced hyperplasia and adenomas. Caloric restriction significantly decreased levels of cytosolic testicular 15-, 5-, and 12-lypoxygenase activities by 78, 12, and 69%, respectively in 1 year old Fischer 344 rats and by 70, 53 and 55%, respectively in 1 year old Brown Norway rats. Flutamide (15 mg/kg/day, p.o., 3 months) significantly increased the severity of hyperplasia in *ad libitum* Fischer rats from 1.9 to 3.9 on a scale of 1-4, while caloric restriction decreased the severity in flutamide treated rats to 0.4. The percent of rats with Leydig cell hyperplasia flutamide-treated rats (1 year old) decreased from 100% in *ad libitum* fed animals to 42% in calorically restricted animals. These results indicate that caloric restriction protects against flutamide-induced testicular tumors possibly by decreasing testicular lypoxygenase activity. (Supported by AICR Grant #NC92B03 and the NCTR.)

622 THE REQUIREMENT AND TOXICITY OF REACTIVE OXYGEN SPECIES IN MOUSE GAMETE FERTILIZATION

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Although reactive oxygen species are essential for fertilization, excess amounts of hydrogen peroxide are detrimental to the fertilizing ability of mouse gametes. In a previous study, reduction in fertilizing ability in bisdithiocarbamate treated mice was associated with decreased production of reactive oxygen species in epididymal spermatozoa. In this follow-up study, the necessity for reactive oxygen species in fertilization was examined. Glucose, as the sole carbohydrate source, supports capacitation and fertilization of mouse gametes *in vitro*. In the presence of glucose alone, 78.6 ± 7.6% of the eggs were fertilized. Without glucose, despite the presence of other carbohydrates, no eggs were fertilized. When glucose was substituted with fructose or fructose-6-phosphate, along with xanthine, xanthine oxidase, and catalase (the xanthine oxidase system), maximum fertilization was obtained. The results indicated that the xanthine oxidase system, providing the exogenous reactive oxygen species for sperm fertilizing ability, could also be toxic to gametes in the absence of catalase. When catalase was eliminated, the substitution supported only 13.5 ± 3.3% fertilization. Results from this study support the hypothesis that reactive oxygen species are essential for the normal progress of sperm capacitation while excess hydrogen peroxide is detrimental to fertilization. Therefore, xenobiotics may decrease sperm fertilizing ability by either increasing or decreasing the production of reactive oxygen species. [Supported by NIEHS P42-ES04911-05]

623 STEM CELL FACTOR REPLETION STIMULATES THE RECOVERY OF 2,5-HEXANEDIONE-INDUCED TESTICULAR ATROPHY

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2,5-Hexanedione (HD) exposure causes an irreversible testicular atrophy in rats characterized by seminiferous tubules containing Sertoli cells, stem germ cells and only immature spermatogonia long after toxicant withdrawal. The current study is focused on describing the mechanism of this testicular atrophy. Histopathological analysis along with the presence of low molecular weight DNA ladders and DNA fragmentation *in situ*, identified that the HD-dependent germ cell loss was the result of apoptosis. One common mechanism which results in apoptosis is growth factor disruption. Stem cell factor (SCF) is a growth factor essential for progression of spermatogonia into spermatocytes and also protects spermatogonia from apoptosis *in vitro*. SCF is expressed in two forms; one which is released as a soluble form and one which remains associated with the cell membrane. The form of SCF expressed is determined by alternate mRNA splicing which was measured by the reverse transcription-polymerase chain reaction. Following HD exposure, the normal expression of testis SCF mRNA is disrupted in a time-dependent manner correlating with the germ cell loss and irreversible atrophic injury. Infusion of HD-induced atrophic testes with SCF continuously for 2 weeks increased germ cell proliferation suggesting SCF repletion stimulated recovery. Together, these data support a role for SCF disruption as a mechanism involved in the long-term atrophy observed following HD exposure.

624 OVARIAN LUTEAL CELL TOXICITY OF 2-METHOXY ACETIC ACID

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We proposed that the ovarian luteal cell is a primary target of ethylene glycol monomethyl ether (EGME) in the female rat because gavage doses of EGME significantly increased serum progesterone levels, corpora lutea size, and induced a pseudopregnant state in adult cycling female Sprague-Dawley rats. To confirm this conclusion and to test the hypothesis that the increase in progesterone was dependent on cAMP, the effect of the active metabolite of EGME, 2-methoxy acetic acid (MAA), was examined in rat luteal cell cultures. Luteal cells were obtained 3 days after 27 day-old Sprague-Dawley rats were treated twice daily with 0.25 IU human chorionic gonadotropin (hCG) for two days and a subsequent dose of 25 IU hCG. Cells were cultured with or without luteinizing hormone (LH) and various concentrations of neutralized MAA from 1 to 48 hours. MAA (1–25μM) significantly stimulated progesterone production, with or without LH stimulation, in a concentration-dependent manner after 48 hours but not before. Cyclic AMP levels were comparable to controls at all concentrations of MAA and at all time points. However, cellular ATP declined in a concentration-dependent manner contrasting the increasing levels of progesterone. Thus, MAA increased progesterone production, but this increase was independent of cAMP and occurred with declining ATP levels. These results confirm that the luteal cell is a target of EGME in the female, but that the increased progesterone may be a manifestation of luteal cell toxicity rather than a physiological effect. Supported by ES00233, NIEHS, NIH.

625 SERTOLI CELLS MEDIATE GERM CELL APOPTOSIS INDUCED BY 2-METHOXYETHANOL

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Germ cell apoptosis induced by 2-methoxyethanol (2-ME) requires an intact seminiferous tubule. The cell death can be blocked by co-administration of verapamil in vivo. In the present studies, we used adult and juvenile cultured seminiferous tubules (STs) to further explore the mechanism of this lesion. Cultured STs showed germ cell death 19 hr after a 5-hr exposure to 5 mM methoxyaceitic acid (MAA). The lesion was unaffected by the presence or absence of extratubular Ca^{2+}. However, the lesion was prevented by co-treatment with compounds that inhibit Ca^{2+} movement through plasma membranes: verapamil, nifedipine, nicardipine, as well as TMB-8 (non-specific Ca^{2+} movement inhibitor). But inhibitors of Ca^{2+} release from intracellular stores (ryanodine, dantrolene, cyclosporin A) did not block the lesion. Inhibition of calcium mobilization through the IP_3 pathway did not block the MAA-induced lesion. These results suggest that intercellular Ca^{2+} fluxes between germ cells and Sertoli cells mediate the MAA-induced germ cell death. Previous reports noted an increased frequency of stage XIV tubules in 2-ME-treated rat testes. We re-evaluated the frequency of stage XIV tubules both in testes in vivo and in cultured STs in vitro. Both in vivo and in vitro exposures increased the frequency of stage XIV tubules. Based on these data, we propose that 2-ME/MAA produces an initial effect on Sertoli cells that increases Ca^{2+} movement into germ cells, which perturbs cell cycle, and kills the spermatocytes. The structures that mediate this increased calcium flux are under investigation.

626 PREIMPLANTATION EFFECTS OF AMMONIUM DINITRAMIDE (ADN) ADMINISTERED IN THE DRINKING WATER OF SPRAGUE-DAWLEY RATS

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The Department of Defense is evaluating ammonium dinitramide for use as a solid rocket propellant and as an explosive. Previous studies have shown that ADN is a reproductive toxicant, causing implantation failure in Sprague-Dawley rats when it is administered during the preimplantation period of gestation. This study was designed to evaluate the implantation failure. After successful mating, female rats were treated with 2.0 grams/liter ADN in their drinking water for 24, 48, 72, or 96 hours before preimplantation embryos were harvested from the oviducts or uterine horns. The stage of development of the embryos was evaluated by phase contrast microscopy. On gestation day one, 10.2 ± 0.48 (SEM) and 10.3 ± 0.42 morphologically normal 2-cell embryos were harvested from the treatment and control groups, respectively. On gestation day two, the development of the embryos harvested from the treated animals was either slowed or halted when compared to the control embryos. By gestation day four, 10.3 ± 0.49 embryos from control animals were harvested from the uterine horns; these had developed to the late morula or blastocyst stage. On gestation day four in the treated group, 7.3 ± 1.4 developmentally halted (4-cells or less) or degenerate embryos were collected from the oviducts (82%) or uterine horns. These data suggest that the implantation loss seen in animals treated with ADN is due to embryolethality. (Supported by DOD Contract No. F33615-90-C-0532)

627 A HISTOPATHOLOGICAL AND CYTOLOGICAL EVALUATION OF BOVINE CORNEAS EXPOSED TO ANIONIC OR CATIONIC SURFACTANTS IN A BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY

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In this study, we determined the histological differences of bovine corneas after exposure to two types of surfactants. Bovine corneas were exposed to a 10% aqueous solution of dioctyl sulfo-succinate (DOSS) or a 0.5% aqueous solution of Benzalkonium Chloride (BAK) in the same manner as in the Bovine Corneal Opacity and Permeability Assay (BCOP). Control corneas were exposed to Minimal Essential Medium (MEM) only. Opacity determinations were made 10 minutes and two hours after exposure. A permeability assay was not performed. Following the opacity determinations, the corneas were removed from the holders and fixed in 10% neutral buffered formalin for histopathologic evaluation. Cytologic evaluation of the MEM in the anterior chambers of the BCOP holders was also performed. Corneas exposed to DOSS had moderate to severe sloughing of the most superficial layers of corneal epithelium. After 10 minutes of exposure, sheets of cells were partially separated from the corneas and at 2 hours after exposure the superficial layers of epithelium were largely absent. Corneas exposed to BAK had moderate to severe edema of the middle and basal layers of the epithelium. Ten minutes after exposure to BAK moderate edema of the middle layers of the cornea was noted. Two hours after exposure severe diffuse edema with vacuolation and partial separation was noted. Cytological evaluation revealed corneal epithelial cells in the chamber fluid from corneas exposed to DOSS for 2 hours, but not in the other preparations.

628 VALIDATION OF THE BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY (BCOP) IN PREDICTING EYE IRRITATION OF PHARMACEUTICALS AND REACTIVE INTERMEDIATES

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A validation study to evaluate the BCOP assay as a predictive screen for eye irritation was applied to 61 chemicals (32 reactive intermediates and 29 active pharmaceuticals) and the results compared to eye irritation studies with rabbits. Preliminary studies indicated that a net opacity value (at 570 nm) of 0.95 or greater or a net fluorescein permeability value (at 490 nm) of 0.6 or greater predicted corneal effects of active pharmaceuticals. In the present study, corneal effects were correctly predicted for 76% of the active pharmaceuticals tested and incorrectly predicted for 24% (all false negatives). Use of the BCOP was relatively predictive for corneal effects, did not result in any false positives and would result in a reduction of the use of rabbits. For reactive intermediates, corneal effects were correctly predicted for only 56% of the compounds tested and incorrectly predicted for 44% (34% false negatives, 9% false positives). Use of the BCOP for reactive intermediates was not predictive for corneal effects, resulted in a large incidence of false negatives and positives and would not lead to a reduction in the number of rabbits used. Based on these results, the BCOP appears to have potential as a screening tool for corneal effects for active pharmaceuticals but not for reactive intermediates.

629 A COMPARISON OF *IN VITRO* EYE IRRITATION TEST DATA FOR ORGANOSILICON COMPOUNDS

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There are increasing efforts towards the development of alternatives to in vivo eye irritation testing in animals for humane, scientific and economic reasons. Two commercially available *in vitro* assays (Epi-Ocular Tissue Model OCL-100 and Skin^{2}™ 1200 Model) and a third published assay, the Bovine Corneal Opacity and Permeability Assay (BCOP), were used to assess the eye irritancy potential of several organosilicon (OS) compounds for the purpose of evaluating their suitability for testing silicone polymers and other materials. The Skin^{2}™ 1200 Model (9 OS compounds) was generally applicable for the *in vitro* assessment of eye irritation of liquid silicone polymers, however some effort will be required to overcome the problem of working with viscous pastes and volatile low surface tension test materials for which variable results were observed. The correlation of the Skin^{2}™ 1200 Model *in vitro* eye irritancy results with known *in vivo* data were generally good for silicone polymers, although some alkoxysilanes do not appear to give *in vitro* results which correspond with their *in vivo* irritancy. *In vitro* results obtained with the Epi-Ocular Tissue Model OCL-100 (although limited), correctly identified both irritant and non-irritant silicone polymers. The BCOP assay correctly identified 4 non-irritant (2 polydimethylsiloxanes, 1 phenylsilsesquioxane and 1 silicone polyether) and 2 irritant aminofunctional siloxane polymers which are extensively used in personal care formulations; histological examination of the treated corneas yielded results which correlated well with the opacity and permeability end-points. All 3 models performed well and are worthy of future study with OS compounds; of the two tissue construct models, the Epi-Ocular Tissue Model OCL-100 had several advantages over the Skin^{2}™ Model in that it appeared to have a better technical design, facilitating more reliable dosing of the test materials and ease of use.

630 FORMATE-INDUCED INHIBITION OF RETINAL MITOCHONDRIAL ATP SYNTHESIS

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The apparent selective vulnerability of the retina and optic nerve to the neurotoxic actions of formate in methanol poisoning has been the subject of considerable speculation. Formate has been hypothesized to produce retinal and optic nerve toxicity by disrupting mitochondrial energy production. Previous studies in our laboratory have documented formate-induced retinal dysfunction, depletion of retinal ATP and mito-chondrial disruption in a rodent model of methanol intoxication. The purpose of this study was to investigate the effect of formate on ATP production in intact mitochondria isolated from two highly aerobic tissues with apparent differences in formate-sensitivity, retina and heart. Mitochondrial ATP synthesis was measured in the presence of the NAD-linked substrates pyruvate plus malate. Formate produced a concentration-dependent reduction in the rate of ATP synthesis in bovine retinal mitochondria with maximal inhibition observed at 10 mM. ATP synthesis in retinal mitochondria was reduced from a control value of 4.04 ± 0.26 μmole/min/mg of mitochondrial protein to 2.83 ± 0.32 μmole/min/mg in the presence of 10 mM formate. Comparative studies conducted in mitochondria isolated from bovine heart showed no effect of formate (10 mM) on ATP synthesis (control - 1.34 ± 0.23 μmole/min/mg; formate - 1.20 ± 0.26 μmole/min/mg). These findings support the hypothesis that formate acts as a specific mitochondrial toxin in the retina and suggest that one component of the retinotoxic actions of formate may be due to tissue-specific differences in mitochondrial metabolism. (Supported by The American Petroleum Institute and NIH grants RO1-ES06648 and P30-EYO1931).

631 VARIATIONS IN INTRAOCULAR PRESSURE BETWEEN NEW ZEALAND WHITE AND DUTCH BELTED RABBITS

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Glaucoma is a disease complex characterized chiefly by an increase in intraocular pressure that, if sufficiently high and persistent, leads to damage of the optic disc at the juncture of the optic nerve and retina. Irreversible blindness can be the result of such excursions in pressure from the normal range. There are three types of glaucoma, primary, secondary and congenital. Primary glaucoma is further subdivided into narrow-angle and wide-angle types, based on the configuration of the angle between the iris and the inner surface of the trabecular meshwork in the anterior chamber, where reabsorption of the aqueous humor occurs. A large number of chemicals are known to affect the levels of intraocular pressure, and those therapeutic agents that intentionally lower intraocular pressure are referred to as antiglaucoma agents. Such chemicals mediate this pressure lowering effect through a variety of biochemical and physiologic mechanisms. Nonetheless, efforts continue to discover new therapeutic agents in existing classes as well as novel agents to treat elevated intraocular pressure. Typically, the rabbit has been the species that has been considered to be the most sensitive for ocular studies. Our laboratory has found that the intraocular pressure of New Zealand White rabbits is significantly lower than that found in Dutch Belted rabbits, thereby making the latter a much better animal model in which to evaluate the ability of potential antiglaucoma agents to lower intraocular pressure. Data is presented in support of this claim.

632 13-WEEK OCULAR TOXICITY STUDY OF METRONIDAZOLE IN RABBITS

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Metronidazole is being evaluated for potential use topically in treatment of ocular rosacea. The purpose of this study was to assess the toxicity of metronidazole (0.75% solution), when applied as a single drop to the right eyes of male Hra: (NZW) SPF rabbits (n = 8) four times daily for at least 13 weeks; the left eye was not treated. The treated and untreated eye of each animal was examined with an indirect ophthalmoscope and a slit lamp biomicroscope pretest; on Day 1 (1 hour after the third dose); and approximately weekly thereafter (1 hour after the second dose). The slit lamp biomicroscopic evaluations were done with sodium fluorescein staining of the cornea using a modified McDonald-Shattuck scoring system. Each animal was also scored pretest (Day - 1) and daily (just before the second dose) starting on the first day of treatment by laboratory technicians using the Draize technique. After at least 13 weeks of treatment, animals were anesthetized, exsanguinated, and necropsied. Both eyes from each animal were collected, preserved, sectioned and stained for microscopic examination. There were no apparent test material-related observations indicative of local ocular or systemic toxicity. Based on the results of this study, administration of one drop of metronidazole to the right eye of each animal four times daily for at least 13 weeks at a concentration of 0.75% showed no adverse effects in male Hra: (NZW) SPF rabbits.

633 OPHTHALMOLOGICAL EVALUATIONS IN DOGS FOLLOWING SUBCHRONIC ADMINISTRATION OF FORTRESS®, A CHOLINESTERASE-INHIBITING INSECTICIDE

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A subchronic study was conducted in beagle dogs to assess ocular function and morphology and cholinesterase activity following dietary administration of the insecticide Fortress®. Five dogs/sex/group were fed 0, 2, 20, or 60 ppm Fortress® for 6 months. Physical observations, ophthalmoscopic examinations, body weight and food consumption measurements, and cholinesterase activity determinations were performed pretest and at selected intervals during the treatment period. The acute (24 and 48 hr) No Observed Effect Level (NOEL) for inhibition of plasma cholinesterase was 2 ppm. The acute NOEL for erythrocyte cholinesterase inhibition was 60 ppm. There were no treatment-related effects on body weight or food consumption. Clinical signs indicative of peripheral cholinergic stimulation were observed in the 60 ppm groups. Significant depressions in brain and retinal cholinesterase activity were noted in the 20 and 60 ppm males and/or females. No changes in ocular muscle cholinesterase were found. Ophthalmoscopic, electroretinoscopic, tonometric, and light microscopic examinations revealed no definitive functional or morphological ocular changes induced by Fortress®.

634 *IN VITRO* OCULAR IRRITATION ASSESSMENT OF THE CHLORINATED BENZENES

K M E Ng, C P Wang and J I Tu. *Drug Development Division, Development Center for Biotechnology, Taipei, Taiwan.* Sponsor: G M Shivji

Chlorinated hydrocarbons are one of the major environment contaminants worldwidely. Measurement of ocular irritancy caused by environment contaminants is an essential step for sufficient scientific assessment of health risk. In order to develop an in vitro test battery as a pre-screen tool for ocular irritancy of chlorinated compounds, the pentachlorobenzene (PCB), pentachlorophenol (PCP), pentachloronitrobenzene (PCNB) and trichlorotoluene (TCT) were studied. Three cytotoxicity assays [MTT incorporation, neutral red uptake and total protein content determination] were analyzed to assess their values for ocular irritancy prediction. For each test, rabbit corneal cells (SIRC) and NIH 3T3 mouse fibroblast cells were tested. The results of this study demonstrated that chlorinated compounds caused dose- and time-dependent of cytotoxicity. The sensitivity of all these assays with four chlorinated compounds showed a strong correlation revealing the same rank order of irritancy. For SIRC cells, the order of irritancy is PCP≥PCB > TCT > > PCNB; for NIH cells is PCB≥PCP > PCNB > > TCT. These results suggested that SIRC cells may be more suitable for eye irritation prediction of chlorinated compounds than NIH cells.

635 VITAMIN A (VA) POTENTIATION OF VINYLIDENE CHLORIDE (VDC) TOXICITY IN PRECISION-CUT RAT LIVER SLICES

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Previously, our studies demonstrated that VA pretreatment increased VDC hepatotoxicity both *in vivo* and in precision-cut rat liver slices. $GdCl_3$ (10 mg/kg iv) partially reversed VA potentiated VDC hepatotoxicity indicating that Kupffer cells play a role in this potentiation. As VA pretreatment resulted in increased mortality in rats given higher VDC doses, rats (male Sprague Dawley, 250–325 g) were treated with 50 mg/kg VDC ip (in 4 ml peanut oil) 24 hr after oral gavage of VA (250,000 IU/kg). Controls received VA vehicle (H_2O/propylene glycol/tween 20, 83:10:7). In another experiment, rats received 10 mg/kg $GdCl_3$ right after VA and 50 mg/kg VDC 24 hr later. Blood was obtained from abdominal aorta for plasma ALT, and samples of liver were fixed in 10% buffered formalin for histology. VA + VDC resulted in a significant elevation of ALT (584 ± 153 VA vs 33 ± 5 U/L control) and centrilobular necrosis. $GdCl_3$ reversed the increase in ALT (48 ± 3 U/L) and decreased extent of necrosis. To examine histological changes in liver slices due to VA + VDC, liver slices from VA pretreated rats were exposed to 0.025, 0.05 and 0.1 μl VDC evaporated into a total volume of 25 ml and incubated for 2, 4 and 8 hr, fixed in buffered formalin, and sections were stained with H&E. In VA pretreated liver slices VDC exposure resulted in centrilobular necrosis, the extent of which depended on VDC concentration and time. To examine whether VA has effects on cytochrome P-450 IIE1, which bioactivates VDC, *p*-nitrophenol (PNP) hydroxylation by VA pretreated and control liver slices was compared and the IIE1 content of liver slices was quantified by densitometric analysis of Western Blots. VA resulted in a 2-fold increase in PNP hydroxylation (1097 ± 259 VA vs 520 ± 163 pmole/mg x 15 min control) as well as IIE1 protein content (Intensity, 3.0 ± 0.6 VA vs 1.4 ± 0.3 control). Results indicate that Kupffer cells as well as an increase in P450 IIE1 play a role in VA potentiation of VDC toxicity. (ES05790).

636 THE *IN VITRO* OCULAR IRRITANCY OF LIQUID PROPELLANT XM46 USING NATIVE RABBIT CORNEAS AND ORGANOTYPIC CORNEAL EQUIVALENTS

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This study was designed to establish a dose-response profile for XM46 using the *in vitro* exposure of native rabbit corneas and rabbit corneal equivalents. Gross evaluation of the native rabbit corneas and the corneal equivalents

following controlled irritant exposure revealed that neat, 1gm/ml and 100mg/ml dilutions produced visual evidence of an irritant effect. Tissue isoelectric focusing analysis revealed that the protein profiles of the corneal equivalents were affected at a higher dilution (0.1mg/ml) than the native rabbit corneas (1mg/ml). In addition, tissue isoelectric focusing data indicated that the native rabbit corneas and the corneal equivalents had a similar biochemical response to acute XM46 exposure. The major protein changes for both native rabbit corneas and corneal equivalents were in proteins with isoelectric points (pI) greater than 7. The fact that the principal corneal and corneal equivalent proteins to be effected by XM46 exposure were basic proteins (pI>7) may be important to understanding the mechanism of toxicity and developing effective methods for reducing the ocular irritancy.

637 CATECHOLAMINES AND VENTRICULAR MYOCYTES; AN *IN VITRO* MODEL FOR STUDYING THE CARDIOTOXICITY OF COCAINE

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Adverse cardiac reactions to cocaine (Coc) have been associated with Coc abuse with increasing frequency over the past two decades. Although the exact mechanisms of Coc cardiotoxicity are unclear, one of the pharmacological properties of Coc includes blockage of catecholamine norepinephrine (NE) reuptake. Catecholamine overload in the heart may result in myocardial cell injury. The purpose of this study was to develop an *in vitro* model of ventricular myocytes to assess the potential cardiotoxic interactions of Coc and NE. Cardiac ventricular cells were isolated from 3–5 day old neonatal rat pups, allowed to attach overnight and were then exposed to 0–4.0 μM NE during a growth period (GP) for 2 days. The cultures were then exposed to a combination of NE (0.2, 2.0, 4.0 μM) and 1 mM Coc for 24 or 48hr. Cell viability was assessed by lactate dehydrogenase (LDH) leakage at 24 and 48hrs. Cell morphology was also noted during the same time periods. Cultures that were not treated with NE during the GP (naive cultures) were more responsive to the cardiotoxic interactions of NE and Coc, as reflected by a 23 to 40% increase in LDH leakage over Coc exposure alone. In contrast, cultures that were exposed to NE during the GP did not demonstrate as much LDH leakage as the native cultures. Vacoulation and granulation was present in all cultures exposed to Coc ± NE at 24hr. Retraction of the cell membrane, along with heavy vacoulation and granulation, were seen in all treated cultures at 48hrs. Spontaneous contractions were conserved in cells cotreated with NE + 1mM Coc, compared to cells with Coc alone. Ventricular myocardial cells in culture combined with adrenergic stimulation may be valuable tools in elucidating the mechanism of Coc toxicity. (This work was supported by NIDA grant# RODA07019).

638 AN *IN VITRO* MODEL FOR STUDYING THE METABOLISM AND BIOACTIVATION OF XENOBIOTICS IN CANINE OLFACTORY MUCOSA

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The olfactory mucosa contains high levels of various xenobiotic-metabolizing enzymes that interact with each other to metabolize inhalants. To explore the possibly complex metabolism of inhalants, an *in vitro* model using canine nasal mucosa explants was developed. Explants were placed on tissue metabolism grids — metal screens held above the bottom of a shallow dish — so that media contacted only the lower surface of the explant. The viability of the explants was examined using flame atomic absorption spectroscopy to quantify K^+ content in previously frozen tissues during incubation at 37°C under 5% CO_2 in Ham's F-12 media. The K^+ content decreased by 50–60% over the first 60 min but was stable between 60 and 120 min. The metabolism of the lipophilic polycyclic aromatic hydrocarbon, benzo[a]pyrene (BaP), by the explants was also examined. ^{3}HBaP (~0.2 pmol) was applied to the lumenal surface of the explants. Following 20- or 60-min incubations, ^3H_2O (a general indicator of BaP metabolism) and covalently bound BaP metabolites (an indicator of the presence of reactive species) were determined. ^3H_2O was separated by vacuum distillation and measured; covalently bound tritium was determined after extracting unbound radioactivity from the tissue with ethyl acetate: acetone (2:1) and oxidizing the remaining tissues while collecting the released ^3H_2O for analysis. Explants from five Beagle dogs converted 5.9×10^{-4} to 9.3×10^{-4} pmol (0.42–2.54% of the dose) to tritriated water and covalently bound 3.3×10^{-4} to 2.5×10^{-3} pmol (0.24–6.8% of the dose). These data suggest that a significant portion of inhaled environmental pollutants is metabolized by the nasal mucosa. [Supported by NIH Grant ES05910 from NIEHS in facilities provided by the U.S. DOE/OHER under Contract No. DE-AC04-76EV01013.]

639 METABOLISM OF ^{14}C-1,3,5-TRINITROBENZENE (TNB) *IN VITRO*

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TNB is a class A explosive that is less sensitive to impact but more powerful and brisant than 2,4,6-trinitrotoluene (TNT) and is used primarily in explosive compositions and munitions. It is also a photodegradation product of TNT and has been detected as an environmental contaminant of surface water, ground water and soil near TNT production waste disposal sites and at certain U.S. Army installations. TNB has been shown to undergo biotransformation to 3,5-dinitroanaline in microbial systems. There is no information on the metabolism of TNB *in vitro* in mammalian system. We studied the metabolism of ^{14}C-TNB with Fischer 344 male rat liver microsomal enzymes. The incubation test tubes containing a reaction mixture of: $MgCl_2$ 5 mM; NADPH 2.5 mM; $NADP^+$ 2.5 mM; glucose-6-phosphate 12.5 mM; glucose-6-phosphate dehydrogenase 2 units; ^{14}C-TNB 1μCi 100 μM; and 1.5 to 2 mg microsomal protein; in 2 ml of Tris-HCl buffer (66mM, pH7.4) were incubated for 5 minutes in a shaking water bath at 37°C. The reaction was stopped with 2 ml of methanol. After centrifugation, 10–30 μl of the supernatant fraction was analyzed by reverse phase HPLC with simultaneous UV absorption and radioisotope detection. Two major and one minor metabolites were detected. The peaks were identified as 3-amino-5-nitroaniline (3,5-diaminonitrobenzene) and 3,5-dinitroaniline by either spiking or coelution with authentic standards. TNB was not detected at 5 minutes, however, incubation samples stopped at 1 to 3 minutes showed the presence of the parent compound (TNB). The identification of the minor metabolite and confirmation of these metabolites is in progress.

640 EXAMINATION OF PERCUTANEOUS PENETRATION OF SEMI-PERMANENT HAIR DYES *IN VITRO* USING HUMAN CADAVER SKIN

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In order to estimate the level of systemic exposure to hair dyes, it is necessary to assess the degree of percutaneous penetration of these compounds under use conditions. The percutaneous penetration of six ^{14}C-labeled semi-permanent hair dyes (HC Orange No. 1, HC Red Nos. 1 and 3, HC Yellow Nos. 4 and 15, and Disperse Black 9), representative of diphenylamine, nitrophenyl and azo chemical classes, was examined *in vitro* using female breast and abdominal cadaver skin. Heat-separated epidermal skin sections (n = 3–5 donors) were mounted in- static Franz-type diffusion cells. Each labeled dye was incorporated in a commercial hair dye base at a level of 1 to 1.5% (w/w). Dye formulations (10 mg/cm^2) were applied to the surface of the skin for 30 minutes to simulate typical use conditions. Residual dye material was then rinsed from the donor chamber and the receptor fluid was analyzed for the presence of ^{14}C over a 48 hour period. Mass balance (rinse, terminal wash, skin, receptor fluid) was also determined. Mean cumulative percutaneous absorption levels (ug/cm^2) were 1.56, 1.49, 0.24, 0.15, 0.04, and 0.03 for HC Red No. 1, HC Orange No. 1, HC Red No. 3, HC Yellow No. 4, HC Yellow No. 15 and Disperse Black 9, respectively. In separate experiments, ^{14}C-HC Yellow No. 4 was applied together with a number of unlabeled dyes to simulate a shade formulation; the cumulative absorption was reduced by 42%. These results indicate that chemical class alone may not be entirely predictive of the level of percutaneous absorption of semi-permanent hair dyes and the degree of penetration of a single dye may be affected by the presence of others.

641 A METHOD FOR THE DETECTION OF PHOSGENE ($COCl_2$) AND ^{13}C-CARBON DIOXIDE ($^{13}CO_2$) PRODUCED FROM MICROSOMES EXPOSED TO CHLOROFORM ($CHCl_3$) AND $^{13}CHCl_3$

J J Falchi and C L Waller. *ETD/NHEERL, US EPA, RTP, NC.* Sponsor: L S Birnbaum

Chloroform is believed to be oxidatively metabolized to CO_2 via $COCl_2$. We have developed methodology to detect these products following *in vitro* metabolism. Validation of existing physiologically-based pharmacokinetic (PBPK) models could be improved by the detection and quantitation of $COCl_2$ and CO_2. Therefore, the significance of this methodology relies on its potential

application for mass-balance information pertaining to metabolite production resulting from microsomal or other *in vitro* exposures (S-9 fractions, cultured cells, tissue slides) to $CHCl_3$. Using a novel approach which includes the use of a 2-(hydroxymethyl)-piperidine (2-HMP) coated silica trap suspended over a microsomal preparation containing male Fischer-344 rat hepatic microsomes, NADPH, and $CHCl_3$, the reactive metabolite $COCl_2$ was produced and trapped as a derivative (1-aza-8-oxa-bicyclo-[4.3.0.] nonan-9-one). The derivative was then identified using gas chromatography/mass selective detection (GC/MSD). In a separate reaction vial, male Fischer-344 rat hepatic microsomes were exposed to $^{13}CHCl_3$ for the production and detection of $^{13}CO_2$. A sample of the head space volume within the reaction vial was taken and the CHCl3 metabolite, 13CO2, was identified using GC/MSD. This methodology offers the ability to derive quantitative information pertaining to oxidative $CHCl_3$ metabolism which could lead to improved mechanistic based risk assessments. (This abstract does not necessarily reflect EPA policy.)

642 GENISTEIN INHIBITS TYROSINE KINASE ACTIVITY AND N-MYC EXPRESSION IN NEUROBLASTOMA CELLS

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Neuroblastoma is a neural crest derived tumor of early childhood. The protooncogene N-myc is overexpressed in a vast majority of neuroblastoma. Evidence from other systems has shown a connection between activation of tyrosine kinase and increased expression of myc oncogene expression. Genistein is an isoflavone found in soy and a known inhibitor of protein tyrosine kinase. We have previously shown that genistein inhibits cell proliferation of neuroblastoma cells and induces differentiation of these cells in a time and dose dependent manner. We further studied the effects of genistein on protein tyrosine kinase activity and N-myc oncogene expression. We found that genistein inhibits the activity of tyrosine kinase and IGF stimulated tyrosine kinase activity in a dose dependent manner. We also found that genistein treatment of neuroblastoma cells decreases N-myc expression. Thus, genistein may be a good candidate for a chemopreventive and/or therapeutic agent against neuroblastoma.

643 COMPARATIVE ANALYSIS OF THE CHRONOLOGY OF PATULIN- AND GOSSYPOL-INDUCED CELLULAR INJURY: MULTIPARAMETER FLUORESCENCE KINETIC ANALYSIS

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Kinetic analysis of a series of endpoints in a clonal rat liver line were monitored using vital fluorescence assays to identify the chronology of injury caused by two food-borne toxins, patulin and gossypol. Cells were exposed to concentrations of patulin (0, 1.0, 10, 100, and 1000 μM) or gossypol (0, 1.0, 3.0, and 10 μM) above and below the IC_{50}. Intracellular glutathione (GSH) content and intracellular Ca^{2+} ($[Ca^{2+}]_i$) were monitored using fluorescent probes requiring uv excitation (351–363 nm); reactive oxygen species (ROS) production, mitochondrial and plasma membrane potential (MMP and PMP), and intracellular pH were monitored with visible wavelength probes (488 nm). Changes in gap junction-mediated intercellular communication (GJIC) were monitored using the gap FRAP technique. All parameters were monitored directly after addition of toxin for 20 min. The analyses provided a distinct chronology of cellular injury. The chronology for patulin was: *simultaneous suppression of GJIC and GSH depletion* ⇒ ROS generation ⇒ mitochondrial membrane depolarization ⇒ simultaneous increase in $[Ca^{2+}]_i$ and cytoplasmic acidification ⇒ depolarization of plasma membrane. For gossypol, the chronology was: *simultaneous suppression of GJIC and generation of ROS* ⇒ cytoplasmic acidification ⇒ simultaneous elevation of $[Ca^{2+}]_i$ and partial depletion of GSH ⇒ mitochondrial membrane depolarization ⇒ depolarization of plasma membrane. This study indicates the utility of these vital assays as improved mechanistically-based methods for hazard identification. (Supported by NIH P42-ES04917 and a TAES Research Enhancement Project).

644 EVALUATION OF RAT LIVER SLICES WITH FLUORESCENCE CONFOCAL MICROSCOPY

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Advances in scanning laser cytometry coupled with computer-based fluorescence imaging of specific fluorophores have facilitated the ability to delineate complex multicomponent and multienzymatic processes within individual cells. This technique, coupled with the use of specific fluorescent probes and substrates, enables both the visualization of a specific macromolecule or cellular component, and quantification with extreme sensitivity. Confocal microscopy coupled with precision cut liver slices will address the intercellular heterogeneity and regional differences in activity within the liver. Initial viability studies were conducted with a molecular probe which is a 3,000 MW polyanionic fluorescein dextran complex to label viable functioning cells. Precision-cut liver slices were prepared from male Sprague-Dawley rats. Cultured liver slices were incubated with vinylidine dichloride (VDC), a potent site-specific toxicant. The dextran was added to the incubation media following a 2 and 4 hr exposure to 1 and 2.5 ul VDC. Distribution of the probe in control slices was uniform indicating viability. The VDC treated slices were nonuniformly labeled with marked disappearance of the viability probe. The slices exposed to 2.5 ul VDC for 4 hr only exhibited small patches of label bordering the portal tract. This confirms earlier histopathology of VDC treated slices. These preliminary results suggest that these techniques can be used for physiological evaluation and direct localization of lesions in precision cut liver slices. (Flinn Foundation, P30-ES-06694).

645 Ca^{2+}-DEPENDENT NUCLEAR AND CYTOSOLIC EVENTS IN A CULTURED MOUSE HEPATOCYTE MODEL OF ACETAMINOPHEN-INDUCED LIVER INJURY

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Acetaminophen (APAP) hepatotoxicity is a process characterized by Ca^{2+} deregulation. Cellular functions utilizing Ca^{2+} as a second messenger affect both cytosolic and nuclear signal transduction. Many independent studies have demonstrated Ca^{2+}-related effects on target molecules in response to toxic doses of APAP, however the primary lesion resulting in liver necrosis has not been determined. We hypothesize that Ca^{2+} -dependent DNA damage is the critical event affecting cell cycle regulation and survival. In this study, DNA single strand lesions were measured by fluorometric analysis of DNA unwinding (FADU) and the status of cytosolic Ca^{2+} was determined by measuring Ca^{2+}-dependent activation of glycogen phosphorylase *a*. Primary cultures of mouse hepatocytes exposed to 7 mM APAP showed a 2-fold increase in % maximum stimulation of glycogen phosphorylase *a* by 6 hr which was reversed with Ca^{2+}-chelating agents. This activity correlated with an increase in LDH release at 6 hours. Cell death was preceded by a decline in % total double stranded DNA after 2 hr, followed by a brief recovery at 4–6 hr. Results from this in vitro model of liver injury are supportive of the hypothesis that the nucleus is a critical target for APAP hepatotoxicity (NIH ES O6345 Minority Supplement).

646 PRIMARY RAT HEPATOCYTES, AN *IN VITRO* MODEL TO STUDY FLUTAMIDE INDUCED CYTOTOXICITY

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Flutamide (FT) has become the antiandrogenic drug of choice for the treatment of prostate cancer. As a result, there has been an increase in the number of cases of flutamide-induced hepatotoxicity. The purpose of this project was to develop an *in vitro* model to investigate the potential hepatotoxicity of FT. Hepatocytes were isolated from 8–12 day old neonatal rat pups and exposed to FT upon confluence, 3 days after plating. FT was evaluated at three exposure periods, 4, 6, and 24 hours, over a concentration range of 10 – 500 μM. Cell viability was assessed by nuclear incorporation of propidium iodide (PI), an indicator of membrane integrity, and 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction, an indicator of mitochondrial integrity. Wavelength scans showed that FT absorbs at 340 nm, which is the peak absorbance level for reduced nicotinamide adenine dinucleotide (NADH) used in the assessment of lactate dehydrogenase (LDH) leakage. Further studies found that FT binds to neutral red (NR), rendering it unavailable for evaluating lysosomal uptake. Therefore, those two classical assays could not be used to evaluate toxicity. Both PI and MTT showed that the hepatocytes responded in a dose dependent relationship to FT. Morphological changes in the hepatocytes were also seen in response to FT exposure. Primary rat hepatocyte cultures may prove to be a valuable model to assess FT toxicity.

647 COMPARISON OF TWO *IN VITRO* METHODS FOR THE DETERMINATION OF ACUTE DERMAL CORROSION POTENTIAL USING OIL PRODUCTION AND REFINERY CHEMICALS

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Two *in vitro* test methods for skin corrosivity, Corrositex® and Skin[2]™, have been approved for use in transport labeling by the US DOT. Evaluations of these *in vitro* test methods have been conducted for chemical families not covered in the initial DOT approvals (e.g., "amines") and for other Nalco/Exxon raw materials and multicomponent intermediates and products. Of the 42 assays conducted using both test methods, 17 of 42 (40.48%) yielded matching results (i.e., same packing group or noncorrosive). In 14 of 42 (33.34%), the Skin[2] results were more severe with 10 of 14 including a noncorrosive determination by Corrositex. In 11 of 42 (26.18%), the Corrositex results were more severe with 6 of 14 including a noncorrosive determination by Skin[2]. Differing test results were not completely attributable to the testing of "amine" containing materials. In five matching tests which also included *in vivo* data, no *in vivo* corrosive material would have been labeled as noncorrosive if the results of both *in vitro* tests were considered. These results suggest that both *in vitro* tests for dermal skin corrosion may be required to ensure confidence in appropriate product labeling and hazard warnings for multicomponent materials.

648 *IN VITRO* QUINOLONE PHOTOTOXICITY: PROSTAGLANDIN E_2 RELEASE FROM 3T3 FIBROBLASTS

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Photosensitivity induced by quinolone antibacterial agents is a serious problem in humans. We previously demonstrated that quinolone administration followed by ultraviolet-A (UVA) irradiation for 4 hr induced inflammation of the auricular skin in Balb/c mice *in vivo*. This change was inhibited by antioxidants and cyclooxygenase inhibitors, suggesting the involvement of reactive oxygen species and cyclooxygenase products in the mechanism. Then we performed the present study focusing on prostaglandin E_2 (PGE_2) and fibroblasts as the cyclooxygenase product and its source, respectively. 3T3 cells were cultured in RPMI 1640 medium including 10% fetal calf serum in 12-well plate for 72 hr until they became confluent. Sparfloxacin (SPFX) or levofloxacin (LVFX) was added to the medium at 1, 10 or 100 μM and irradiated with UVA for 5 min. In another experiment, each UVA-pretreated quinolone was added. The cells were thereafter cultured in quinolone-free medium for 24 hr, and PGE_2 level in the medium was assayed. Simultaneous exposure to SPFX 100 μM and UVA markedly increased PGE_2 level, but UVA-pretreated SPFX did not. LVFX showed no effect at all concentrations in both procedures. The difference in PGE_2 release between 2 quinolones corresponds to the *in vivo* phototoxic potential reported previously. The present results suggest that coexistence of a quinolone and UVA is necessary to induce phototoxicity and that fibroblasts could be a source of PGE_2 to induce skin inflammation.

649 MEASUREMENT OF OCULAR CYTOTOXICITY AND INFLAMMATION USING A BOVINE CORNEAL PUNCH ASSAY

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While the Bovine Corneal Opacity and Permeability assay (BCOP) has proven to be an excellent tool to predict moderate and severe eye irritants, it lacks the desirable sensitivity to distinguish among milder test materials. With assistance from S. Rachui (Stephens & Associates, Carrollton TX) we explored the expanded use of corneal tissue to assess cytotoxicity (as measured by MTT) and inflammatory potential (as measured by PGE_2 release). Our purpose was twofold: 1) employ these concepts to discern between degrees of mildness in products generally classified as mild by the BCOP and 2) to investigate whether cytotoxicity data could be coupled with opacity and permeability to mimic rabbit data generated under FHSA criteria for eye irritation testing. Our first experimentation demonstrated that MTT can be used to rank products by mildness. Test samples included serial dilutions of benzalkonium chloride and three Amway products. The dilution series of each test compound were successfully ranked in order of expected cytotoxicity by MTT and supported visually by the expected dose/response curves. Regarding PGE_2, it appears that it will only be a viable alternative when comparing products that are equally non-cytotoxic. This idea was first presented by Mr. Rachui in a poster at the CAAT Symposium, SOT Conference, Baltimore, MD, 1995. Support to this concept was found in our experimentation where our student researcher was intentionally not informed of Mr. Rachui's presentation yet she still reached the same conclusion on the application of PGE_2 measurement. These results and the prior work of Mr. Rachui add support to using bovine corneal tissue for applications beyond just the BCOP. Application of this concept to FHSA criteria remains the subject of future investigation.

650 EVALUATION OF THE SUSCEPTIBILITY OF CORTICAL AND MEDULLARY REGIONS OF RAT KIDNEY TO CHEMICAL-INDUCED TOXICITY USING TISSUE SLICES

L Gunawardhana, P Ewing, J Cerny, D Clark, D Cugier, B Ennis and M F Miller. *Dept. of Cellular and Microscopic Research, Abbott Laboratories, Abbott Park, IL.* Sponsor: P Cusick

Cortical and medullary regions of the kidney have been shown to be differentially susceptible to the toxicity of a number of chemicals. We have used rat kidney slices to evaluate the susceptibility of renal cortex and medulla to toxicity of S-(1,2 dichlorovinyl)-L-cysteine (L-DCVC, 21.5 and 107.5 μg/ml), gentamycin (100, 250 and 500 μg/ml) and ciprofloxacin (15, 75, 150 μg/ml). The toxicity of these three compounds was evaluated using morphological examination of H & E stained paraffin sections, LDH leakage and K^+ content. Following 24 hr treatment, L-DCVC caused a 30–60% increase in LDH leakage and decreased slice K^+ content by 50–90%. Morphological evaluation of rat kidney slices treated with L-DCVC (21.5 μg/ml) showed injury predominantly in the outer medulla. This injury was characterized by mild to moderate single cell necrosis and moderate to marked hydropic degeneration of renal tubular epithelium. Mild hydropic degeneration of tubular epithelium was also observed in the cortex and inner medulla of these slices. In contrast, the injury caused by gentamycin was localized to the cortical region of kidney slices. Gentamycin caused mild elevations in LDH leakage (15%) and decreased K^+ content by 10–15%. Rat kidney slices treated for 24 hr with ciprofloxacin showed no adverse effects compared to controls. The relative nephrotoxicity of L-DCVC, gentamycin and ciprofloxacin in rat kidney slices correlated well with the relative potencies reported for these compounds *in vivo*.

651 MECHANISMS OF CYTOTOXICITY OF CULTURED RAT HEPATOCYTES BY NAPHTHOQUINONES

Y Fujikawa, K Tsukidate, T Mikami. *Dept of Drug Safety Research, Eisai Co. Ltd., Ibaraki, Japan.* Sponsor: John L. Farber

The relationship between the mechanism(s) of cytotoxicity by various naphthoquinones and their one-electron reduction potentials was studied. The one-electron reduction potentials decrease in the series dichlone(-5mV) > naphthazalin(-50mV) > 1,4-naphthoquinone (1,4-NQ) (-160mV) > menadione(-240mV). Cytotoxic potentials of these quinones to cultured rat hepatocytes were almost similar; the median lethal concentrations (LC50) of these quinones were 20 ~ 40μM. Cytotoxicity of dichlone that have the higher reduction potential than any other quinones was completely prevented by the ferric iron chelator deferoxamine (DFX). DFX reduced by 50% the toxicity of naphthazalin, 1,4-NQ and menadione. However, malondialdehyde was not formed with all quinones and the antioxidant N, N'-diphenyl-*p*-phenylenediamine did not protect the cytotoxicity of all quinones. The calcium chelator EDTA potentiated the cell killing by these quinones. Intracellular ATP content did not alter prier to cytotoxicity of these quinones except menadione. ATP depletion preceded the cell killing by menadione and fructose reduced by 30% the toxicity of menadione.

These results indicate that cytotoxic potentials of these quinones are not related to their reduction potentials. The toxicity of dichlone (the highest reduction potential, -5mV) is completely ferric iron dependent, but lipid peroxidation is not involved in this toxicity, whereas that of menadione (the lowest reduction potential, -240mV) is by both mitochondrial injury and iron-dependent pathway. The toxicity of 1,4-NQ and naphthazalin is partly iron-dependent but there might be another unknown pathway (probably covalent binding).

652 TIMECOURSE OF CYCLOPHOSPHAMIDE TOXICITY TO RAT LIVER SLICES *IN VITRO*

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Cyclophosphamide (CP), a biotransformation-dependent toxicant, was used to determine the potential application of rat liver slices to assess hepatotoxicity of novel therapeutics early in drug development. Precision cut liver slices were prepared from phenobarbital (PB)-induced or control Sprague Dawley rats using the Krumdiek system and maintained in roller culture. Incubation with CP (1uM to 1mM, 2hr) resulted in a dose-dependent depletion of glutathione (GSH) in the absence of cell death indicated by LDH or ALT leakage. Increasing the exposure time to CP (1mM) from 2 to 6hr depleted GSH by 98% in PB-induced slices and 65%–74% in control slices, but significant enzyme leakage was detected only in the PB-induced slices (38 ± 11 % LDH leakage versus 12 ± 2% with vehicle). Extension to 12hr was required to observe enzyme leakage in non-induced liver slices exposed to CP. Morphological assessment of fixed slices by light microscopy indicated little or no necrosis even in PB-induced slices at the earlier time points. These observations are consistent with previous studies that show CP is metabolized by PB-inducible cytochrome P450s to cytotoxic alkylating metabolites. GSH was the earliest parameter perturbed followed by enzyme leakage and finally morphological changes. These observations impact the design of slice experiments where exposure time and indices should be carefully chosen.

653 REPRODUCIBILITY AND CORRELATION OF EPIOCULAR, A THREE DIMENSIONAL TISSUE CULTURE MODEL OF THE HUMAN CORNEAL EPITHELIUM

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The EpiOcular™ tissue model is an organotypic model of the human corneal epithelium (HCE) cultured from normal human keratinocytes using serum free medium. Paraffin embedded, H & E stained histology cross-sections show the structure of EpiOcular closely parallels that of the HCE; large nucleated basal cells lie beneath 5–6 stratified cell layers which progressively flatten out, ending with a highly squammous, non-keratinized layer at the culture's apical surface. Over the first 3 months of commercial production, 34 lots of EpiOcular have been produced and monitored using the MTT assay. Exposure of a common surfactant, 0.3% Triton X-100, gave Effective Time-50 values (time of exposure after which viability is reduced to 50%, or ET-50) which had an average of 32.6 ± 9.4 minutes (± 1 standard deviation). In addition, ET-50 values were correlated to the Draize rabbit eye scores for 17 common chemicals from the ECETOC ocular irritancy data base and 13 final formulation shampoos and gave correlation coefficients, r, which were 0.922 and 0.856, respectively. Finally, release of the inflammatory mediators, interleukin 1-alpha and prostaglandin E-2, has also been observed following treatment of EpiOcular with mild surfactants such as Tween 20. These initial results provide the basis for further investigating the use of the EpiOcular tissue model to reduce and/or replace in vivo, animal based ocular irritancy testing.

654 ALTERATION OF OXYTOCIN-INDUCED CALCIUM OSCILLATIONS IN CLONE 9 CELLS BY TOXIN-EXPOSURE

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A communication-competent clonal rat hepatocyte cell line (Clone 9) is being developed as a model system to investigate the action of toxins on amplitude and frequency encoded $[Ca^{2+}]_i$ derived from extracellular signals. Clone 9 cells exhibit an initial $[Ca^{2+}]_i$ spike followed by Ca^{2+} oscillations for at least 30 minutes after exposure to oxytocin (10 to 100 nM). The initial Ca^{2+} peak is suppressed by nifedipine and oscillations are blocked by thapsagargin pretreatment. Oscillations are also accompanied by a gradual decrease in mitochondrial membrane potential (MMP) as determined using the potentiometric fluorescent probe, rhodamine 123. Brief exposure of cells to toxin (patulin, gossypol or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) at doses which have no effect on cell viability were used to monitor any changes in oxytocin responsiveness of cells. Toxin treatment either blocked or changed the amplitude and/or frequency of Ca^{2+} oscillations depending upon the toxin-induced chronology of cellular injury. Mitochondria exhibited a sharp drop in membrane potential directly after addition of oxytocin. These studies indicate that toxic agents may alter amplitude and frequency encoded information derived from cell signaling events which could result in altered cellular homeostasis at nonlethal doses of toxin. (Supported by NIH P42-ES04917 and a TAES Research Enhancement Project).

655 FURAN-MEDIATED DNA DOUBLE STRAND BREAKS IN ISOLATED RAT HEPATOCYTES

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Furan is a potent non-mutagenic, non-DNA reactive rodent carcinogen that produces hepatocellular carcinomas in male Fischer 344(F-344) rats. Furan-induced carcinogenesis appears to be related to cytolethality followed by cell proliferation and ultimately tumor formation. However, the relationship between cytolethality, cell proliferation, and tumor formation is not known. Previous studies in our laboratory have shown that exposure of isolated rat hepatocytes to toxic concentrations of furan produced decreases in ATP concentrations that preceded cytotoxicity (Mugford and Kedderis, 1995, *Toxicologist* **15**: 283). Other investigators have demonstrated that several cytolethal non-DNA reactive carcinogens that deplete ATP produce DNA double strand breaks (dsb) prior to cell death (Elia *et al.*, 1994, *Environ. Molec. Mutagen.* **24**: 181–191). The present studies were conducted to determine if exposure to furan produces DNA dsb in isolated rat hepatocytes. Hepatocytes were isolated from male F-344 rats and incubated with furan (100 μM) for 0–4 hr at 37 °C. Following incubation, an aliquot of cells from each suspension was encapsulated in agarose plugs and processed for pulsed field gel electrophoresis. Plugs were loaded into 0.8% agarose gels and run for 23 hr on a Bio-Rad CHEF mapper system. Gels were stained with ethidium bromide and analyzed under UV light. The amount of DNA migrating onto the gel from each well was quantitated using a video image analysis system. Furan-mediated DNA dsb were detected as early as 30 min after exposure and remained elevated. The formation of DNA dsb after furan treatment may be involved in the induction of mutations leading to liver cancer.

656 SILYMARIN REDUCES ETHANOL TOXCITY TO A431, EPIDERMOID CELLS, *IN VITRO*

M G Neuman, I Malkiewicz and N H Shear. *Division of Clinical Pharmacology, Sunnybrook H.S.C., Toronto, Ont, Canada.* Sponsor D N Sauder

In previous studies we showed that the natural flavonoid Silymarin (S) is protective against acetaminophen-induced cytotoxicity *in vitro*. The aim of this study was to measure the effect of EtOH in the presence or absence of S on epidermal cell viability and interleukin (IL)1-α in modulation of toxicity and protection. A431 epidermoidal cells were plated in six 96 wells tray to 80% confluence in the complete growth medium. Cells were incubated with 80 mM EtOH, α-MEM (control/C), 500 μM S or 500 μM S + EtOH concomitant, for 24 hours. Cytoviability was measured by reduction of tetrazolium dye (MTT), and ELISA was used to measures IL1-α into media. ANOVA for multiple treatments was used to calculate the significance.

The viability of control (untreated cells) is 100%. S does not affect the viability (102%), while EtOH reduces the viability to 57% ($p<0.0001$ vs C). Cells treated with EtOH and S have a viability of 89% ($p<0.0001$ vs EtOH treated). IL1-α (pg/ml) realease into media are as follows: control 8.25, S 12.25, EtOH 47,75 (increase $p<0.0001$ vs control, EtOH + S 13,25 (decrease $p<0.0001$ vs EtOH).

We conclude that EtOH is toxic to epidermal cells. S reduces this toxicity. EtOH significantly increases cytokine release into media and S prevents this effect.

657 USE OF PRECISION-CUT LIVER SLICES FOR INTERSPECIES COMPARATIVE TOXICITY

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Compound A is a novel tissue-selective potassium-channel activator that was not hepatotoxic in dog, monkey and rat preclinical studies, whereas this compound induced elevated serum liver transaminases in Phase II clinical trials. Compound B, the back-up candidate, has not been evaluated in clinical trials. The objectives of this study were to utilize human, monkey and rat precision-cut liver slices to: 1) compare interspecies hepatotoxicity with Compound A and B and, 2) determine if this *in vitro* assay is predictive of *in vivo* events. Endpoints measured after 24 hours exposure to test article included protein synthesis, protein secretion, ATP content and LDH leakage. Compound A inhibited protein synethesis at significantly lower concentrations

in all species as compared to Compound B. Compound A inhibited protein synthesis at significantly lower concentrations in human liver slices as compared to monkey or rat, similar to the interspecies differences of hepatotoxicity *in vivo*. The results of this study indicate that the *in vitro* precision-cut liver slice may be a reliable system for predicting interspecies comparative toxicity *in vivo*.

658 IN VITRO ASSESSMENT OF BIOLOGICAL ACTIVITY OF MAINSTREAM SMOKE FROM CIGARETTES WHICH BURN OR PRIMARILY HEAT TOBACCO

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Smoke from Kentucky reference low "tar" (1R4F) and ultra-low "tar" (1R5F) cigarettes and a new prototype cigarette, which primarily heats tobacco was evaluated for cytotoxic and genotoxic potential using cellular smoke exposure technology (CSET). CSET includes a computer controlled 30 port AMESA/Battelle-Geneva smoke generator which is identical to the system utilized for animal studies and allows cultured mammalian cells (CHO) to be exposed to whole smoke. Cytotoxicity was assessed using the neutral red assay and genotoxicity was assessed using the sister chromatid exchange (SCE) assay. Compared on a per cigarette basis, mainstream smoke from 1R5F and the new cigarette was significantly less cytotoxic and genotoxic than the smoke from the 1R4F cigarette. The cytotoxic and genotoxic potentials of smoke from the 1R5F and new cigarettes were similar. In conclusion, in these assays mainstream whole smoke of a new cigarette had similar cytotoxic and genotoxic potential as an ultra-low "tar" reference cigarette and significantly less activity compared to the whole mainstream smoke from a low "tar" reference cigarette.

659 UPTAKE AND TOXICITY OF $HgCl_2$ AND SYNTHESIZED $Hg(SG)_2$ AND $Hg(CYS)_2$ IN ISOLATED RABBIT RENAL PROXIMAL TUBULE SUSPENSIONS

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Inorganic mercury is known to react with endogenous sulfhydryls, such as glutathione (GSH) and cysteine (CYS), to form mercury complexes, which may be delivered to the kidney and cause toxicity. In order to study this possibility, uptake and toxicity of synthesized $Hg(SG)_2$ and $Hg(CYS)_2$ as well as $HgCl_2$ were investigated in rabbit renal proximal tubule suspensions. The renal proximal tubules were isolated from male New Zealand White rabbit by collagenase digestion followed by purification on a discontinuous Percoll gradient, and were resuspended in incubation buffer containing no additional sulfhydryls. While incubation of tubules alone up to 4–6 hr did not result in the decrease of cellular K^+ content, addition of 10 μM $Hg(SG)_2$, $Hg(CYS)_2$ or $HgCl_2$ caused toxicity within 0.5 hr as assessed by K^+ content. The toxicity rank order of the three mercury compounds was: $HgCl_2 > Hg(CYS)_2 > Hg(SG)_2$. Preincubation of 0.25 mM acivicin, an inhibitor of γ-glutamyltranspeptidase (GGT), had a protective effect on the toxicity of $Hg(SG)_2$. Since the lumen of the isolated tubules has limited access by the Hg compounds, our results suggest that there is basolateral uptake of these compounds and that the toxicity of $Hg(SG)_2$ requires processing to $Hg(CYS)_2$. (NIH P42 ES-04940).

660 INDUCTION OF CYTOCHROME P-450 (CYP) ISOENZYMES IN CULTURED PRECISION-CUT RAT AND HUMAN LIVER SLICES

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In this study we have examined the effect of some inducers on CYP isoenzymes in precision-cut liver slices. Liver slices were prepared from untreated male Sprague-Dawley rats and from samples of human liver (surplus to transplant requirements) with a Krumdieck tissue slicer and cultured in RPMI 1640 medium in a dynamic organ culture system. Western immunoblotting of liver slice microsomes was performed with antibodies to rat CYP1A2, CYP2B1/2, CYP3A and CYP4A. Treatment of rat liver slices for 72 hr with 0.5 mM sodium phenobarbitone (PB), 50 μM β-naphthoflavone (BNF) and 20 μg/ml Aroclor 1254 (ARO) produced significant increases in the metabolism of 7-ethoxyresorufin and/or 7-benzoxyresorufin. Compared to 72 hr control (no test compound) liver slice microsomes, PB induced CYP2B1/2 and CYP3A, BNF induced CYP1A2 and ARO induced CYP1A2, CYP2B1/2 and CYP3A. Treatment of rat liver slices for 72 hr with 0.5 mM methylclofenapate (MCP), ciprofibrate and Wy-14,643 induced palmitoyl-CoA oxidation and CYP4A. In 72 hr cultured human liver slices 20 μg/ml ARO, but not 0.5 mM MCP, induced 7-ethoxyresorufin O-deethylase activity. Neither ARO nor MCP had any effect on palmitoyl-CoA oxidation and microsomal lauric acid 11- and 12-hydroxylase activities. Compared to 72 hr control human liver slice microsomes, ARO induced CYP1A2 and MCP induced CYP4A. These results demonstrate that cultured liver slices may be used to evaluate the effect of xenobiotics on both rat and human CYP isoenzymes. (Supported by a research grant from MSD Research Laboratories, Harlow, Essex, UK).

661 METABOLISM OF COOKED FOOD MUTAGENS BY PRECISION-CUT RAT AND HUMAN LIVER SLICES

J A Beamand[1], C van den Boom[2], D G Walters[1], R J Price[1] and B G Lake[1]. [1]*BIBRA International, Carshalton, Surrey, England and* [2]*Department of Toxicology, Wageningen Agricultural University, The Netherlands*

In this study we have used liver slices to study the metabolism of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) to polar products (separated by HPLC) and to metabolites that bound covalently to proteins. Liver slices were prepared from untreated and Aroclor 1254 (ARO) treated (single i.p. 500 mg/kg dose 5 days prior to necropsy) male F344 rats and from samples of human liver with a Krumdieck tissue slicer. Slices were cultured in RPMI 1640 medium in a dynamic organ culture system. Liver slices from untreated and ARO treated rats were incubated for 6 hr with either 25 μM [^{14}C]PhIP or [^{14}C]IQ. Metabolism of both PhIP and IQ was greater in liver slices from ARO than from untreated rats. Incubations were also conducted in the presence of either 4 mM diethylmaleate (DEM) or 25 μM furafylline (FURA). DEM significantly reduced liver slice glutathione levels, whereas FURA inhibited 7-methoxyresorufin O-demethylase activity. FURA inhibited PhIP metabolism to polar products in untreated and ARO treated liver slices, while DEM enhanced covalent binding in ARO treated liver slices. IQ metabolism in ARO treated liver slices was inhibited by FURA, whereas covalent binding was increased by DEM in untreated and ARO treated liver slices. Both PhIP and IQ were also metabolised to polar and covalently bound products in human liver slices. These results demonstrate that liver slices may be used to investigate pathways of metabolism of cooked food mutagens. (Supported by the UK Ministry of Agriculture, Fisheries and Food).

662 A NOVEL SHIPPING AND PRESERVATION MATRIX FOR HEPATOCYTE CULTURES

S K Al Casey, E Chacon, and D B Garcia. *CEDRA Corporation, Austin, TX*

The use of *in vitro* alternative methods to screen xenobiotic metabolism and toxicological parameters continue to increase. A variety of *in vitro* models are routinely used to characterize xenobiotic metabolism and evaluate pharmacological and toxicological effects. The wide use of hepatocyte cultures as *in vitro* models has been limited due to a number of reasons: special expertise in harvesting and culturing of hepatocytes; short lived cytochrome (CYP) P450 activity in culture; and ethical considerations relative to the sacrificing of animals for the sole purpose of harvesting a few liver cells. We have developed a novel shipping and preservation medium (Vidacyte™) that permits the transportation of hepatocytes in culture plates while preserving CYP 450 enzymatic activities. In this study, liver cells from dogs, monkeys and rats were successfully transported across the United States. Viability and CYP 450 enzymatic activities were monitored before and after shipping. Our conclusion is that this novel preservation and shipping matrix allows for the first time the utilization of hepatocytes from a single animal by multiple laboratories.

™(patent pending)

662a COLIPA IN VITRO EYE IRRITATION VALIDATION PROGRAMME

COLIPA Task Force Methodology Alternatives: Sponsor: D M Bagley

The COLIPA *In Vitro* Eye Irritation Study has been developed to assess the validity of ten *in vitro* methods for predicting the eye irritation potential of cosmetic formulations and ingredients. The tests evaluated in this study are the neutral red uptake assay, fluorescein leakage assay, red blood cell assay,

EYTEX test, the silicon microphysiometer assay, the hen egg test-chorioallantoic membrane, the chorioallantoic membrane vascular assay, neutral red release assay, the tissue equivalent assay, and the pollen tube growth test. The goal was to determine if a selected set of *in vitro* methods are valid replacements for animal tests in determining the eye irritation potential of cosmetic formulation and ingredients. Specifically this programme will determine whether data from alternative methods: 1. Provide acceptable agreement with the modified Maximum Average Score, or 2. Properly predict irritation in the rabbit eye according to any other alternative algorithms. Fifty five cosmetic ingredients and finished formulations were assembled to assess the performance of the methods. Twenty three of the test substance are ingredients commonly found in cosmetic products. The 32 finished cosmetic formulations represent a broad range of cosmetic product classes.

The validity of the methods was determined by assessing whether data obtained from the *in vitro* methods accurately predicted the endpoints defined by the goals of the projects. To have a basis for the assessment of method validity, the participating laboratories provided Prediction Models that define two critical elements relevant to the use of the *in vitro* assays. Firstly, the Prediction Models define the specific endpoints that are obtained from each assay. Secondly, the Prediction Models difine algorithms that describe how to convert each endpoint from the *in vitro* method into a prediction of eye irritation potential. This information was used to provide and objective statistical assessment of whether the *in vitro* method predicts the actual *in vivo* eye irritation potential. If the Prediction Models are correct, it provides evidence that the models are reliable predictors of eye irritation potential. If the Prediction Models are not accurate it suggests that the methods were not valid under the conditions evaluated in the study.

663 CELL CYCLE PROTEIN EXPRESION IN NORMAL HEPATIC CELL PROLIFERATION AND PEROXISOME PROLIFERATOR INDUCED HEPATIC CELL PROLIFERATION

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During recent years, there has been an extensive research focus in the area of cell cycle control in eukaryotes and the relationship that exists between cell proliferation and cancer. It is currently accepted that the cell cycle is governed by signal transduction pathways mediated by complexes of cyclin dependent kinases (CDKs) and their partner cyclin proteins. Inhibition of this pathway is via tumor suppressor and CDK inhbitory proteins. In this study, protein expression of cell cycle mediators, cyclin dependent kinases (CDKs 1,2,4,5), cyclin proteins(A,B,D1-D3,E, and PCNA), tumor suppressor proteins (Rb, p53), and CDK inhibitory proteins (p16, p21, and p27) were examined following 70% partial hepatectomy (PH) or a single dose of WY14,643 over 96 and 48 hour time courses, respectively. The livers were removed, frozen in liquid nitrogen, and stored at -80°C. One gram portions of liver were then homogenized, centrifuged at 9000 x g, and the supernatant (S9) fraction collected and used for further analysis. CDK1 ($p34^{cdc2}$) and PCNA protein levels were quantified by ELISA and were demonstrated to be significantly increased beginning at the 24 hour time point and maximal at 48 hours (6.9-and 3.7-fold for PH and 4.2-and 3.3-fold for WY14,643, respectively). Immunoblot analysis identified differences between physical and chemical stimuli that focused on G1 cell cycle mediators CDKs4, 5, and cyclin D3. CDK inhibitory protein concentrations rose in accordance with induction of DNA synthesis and histone H1 kinase activity. In addition, there were dramatic differences in p53 protein expression patterns in the two experimental systems. Data generated from this study may aid in elucidating the specific hepatocarciongenic signal transduction pathways stimulated by carcinogenic xenobiotics.

664 DIETHYLSTILBESTROL EFFECTS CELL PROLIFERATION MEDIATED BY GROWTH FACTORS IN CULTURES OF NORMAL AND MALIGNANT HUMAN ENDOMETRIAL CELLS

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Previous studies showed that diethylstilbestrol (DES) may act as a tumor promoter in human endometrial stromal cells. In this study we investigated effects of DES treatment on cell proliferation in human endometrial cells as modulated by basic Fibroblast Growth Factor (bFGF) and Transforming Growth Factor-β (TGF-β). Normal human endometrial epithelial and stromal cells were cocultured on collagen and laminin coated dishes. Normal epithelial cells and cancer cell lines (RL95-2 and HEC-1B) were cultured in serum-free medium (OptiMEM/F12 with additives). Cells were treated for 30 hr with 20 ng/ml of bFGF or 0.1 ng/ml of TGF-β in the presence and absence of 10 ng/ml of DES. Then cells were arrested in mitosis by adding colemid (100 ng/ml) and fixed 12 hr later. Cells were stained with propidium iodide and mitoses were counted in 500-1000 cells per group using a fluorescence microscope. The mitotic fraction (MF = % of cells in mitosis) in normal endometrial epithelial cells compared to control cultures were 185.3% and 0% for bFGF and TGF-β treatments, respectively. However, bFGF decreased MF by 46.4% in cocultures compared to control cultures, suggesting epithelial cell proliferation normally is tightly controlled. DES caused a 33.1% increase in MF in normal endometrial stromal-epithelial cocultures. In response to bFGF, DES-treated cocultures showed a greater decrease (59.0%) in MF than cocultures treated with bFGF alone. MF did not change in either RL95-2 or HEC-1B cells following treatment with DES alone or with bFGF. This shows that DES treatment in normal endometrial cells causes proliferation of epithelial cells and alters stromal regulation of growth factor responses in epithelial cells. In cancer cell lines, however, DES does not effect cell proliferation suggesting that growth factor induced effects on cell proliferation may already be dysregulated. Supported by NIH grants CA31733 and ES07017.

665 INHIBITION OF STILBENE ESTROGEN-INDUCED CELL PROLIFERATION THROUGH THE MODULATION OF INSULIN-LIKE GROWTH FACTOR-I RECEPTOR

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We have investigated the effects of stilbene estrogen, diethylstilbestrol (DES), on the proliferative activity and expression of IGF-I receptor in Syrian hamster renal epithelial cells. DES exposure caused both dose- and time-dependent increases in proliferative activity. In the presence of DES, cell growth increased 2–3 fold after 8 days of DES treatment. The maximum stimulation of cell proliferation occurred at 10 nM DES after 8 days of DES treatment. Cotreatment of cells with ICI 182780 (250 nM) and DES resulted in 50% decrease in cell growth compared to DES alone. Treatment of cells with anti-IGF-IR antibody (αIR3, 1 μg/ml) also significantly reversed the growth-stimulatory effects of DES. IGF-I receptor gene expression analyzed by Northern blotting revealed that DES treatment increased the level of IGF-I R mRNA by 2 fold compared to controls. The cotreatment of ICI 182780 strongly inhibited the DES-induced IGF-I receptor gene expression (50% - 60%). Findings of this study suggest the possibility that the stimulatory effect of DES on the proliferative activity of renal epithelial cells may be mediated through the up-regulation of IGF-I receptors.

666 A COMPARISON OF QUANTITATIVE, IMMUNOHISTOCHEMICAL MARKERS FOR CELL-CYCLE SPECIFIC CHANGES IN F344 RATS

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Chemical carcinogenesis in the regenerating rat liver has been directly linked to perturbations in cell-cycle kinetics. However, the majority of multi-stage cancer risk models that account for the growth of cell populations do not incorporate changes in cell-cycle kinetics. In order to estimate these changes and incorporate cell-cycle information into physiologically based pharmacokinetic/ pharmacodynamic models, a comparison of immunohistochemical markers of cell-cycle specific stages was initiated. Twenty-five rats were given a two-thirds partial hepatectomy (PH) while seven rats were grouped as controls. The rats receiving the partial hepatectomy were sacrificed, five per time point, at 10, 15, 27, 35, and 38 hours following surgery corresponding to the G_1, S, and G_2/M cell-cycle stages. Two-hours prior to sacrifice, rats were administered an ip injection of bromodeoxyuridine (BrdU) (100 mg/kg). Immunohistochemical staining of the tissues consisted of proliferating cell nuclear antigen (PCNA), cyclin D1, BrdU, and cyclin B1. Preliminary results based on PCNA staining, reveal a significant movement of cells into G_1 at the 10 hr time-point with S-phase labeling peaking at the 27 hr time-point. Quantitative comparisons will be made between PCNA, which maintains distinct cell-cycle staining, and the remaining antibodies.

667 A BIOLOGICALLY BASED MATHEMATICAL MODEL OF THE EFFECTS OF PARTIAL HEPATECTOMY ON CELL CYCLE KINETICS

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In an attempt to develop a predictive model for experimental carcinogenesis, we are linking PBPK/PD modeling with Ito's Medium Term Liver Foci Bioassay. Thus, a biologically motivated model depicting cell cycle kinetics is introduced. The model includes detailed descriptions of the cell cycle phases and the biochemical control points among them. Two important control points that are incorporated in the model are the G_1/S "start" point and the G_2/M "transition" point. At the start point, the model characterizes the progression of cells from the G_1 to the S phase by the interactions of P_{33} (cdk) with cyclins A and E (Loyer et al. 1994). The binding of cyclin A with P_{33} motivates the cells to go to the S phase while binding with cyclin E keeps the cell in late G_1 phase (Reddy et al. 1994). Continually, cells are prompted to go into the mitotic phase by the phosphorylation state of the Miotic Promoting Factor (MPF). This process is precisely controlled by the balance of two proteins cdc_{25} and Weel (Novak et at. 1993). Ultimately, the model describes the effects that partial hepatectomy will have on the cell cycle kinetics. For this reason, the model outlines the effect of two important factors, TGF_α and TGF_β, on the biochemical control points of the cell cycle phases.

668 REGULATION OF HUMAN TROPHOBLAST CELL PROLIFERATION BY CADMIUM AND THE CALMODULIN INHIBITOR, ZALDARIDE MALEATE: IMPLICATIONS FOR IMPLANTATION

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Cd inhibits trophoblast cell proliferation in first trimester human placental explants (HPE) and JAr neoplastic trophoblast cells. Calmodulin inhibitors also inhibit cell proliferation, and ameliorate selected toxic effects of cadmium. We hypothesize that zaldaride maleate (ZAL), a calmodulin inhibitor, inhibits trophoblast proliferation, but specifically decreases toxicity of cadmium on human trophoblast proliferation. JAr cells, like HPE, proliferate rapidly and produce placental hormones. In JAr cells, 2 and 20μM Cd significantly inhibited proliferation by 18±7.2% and 76±11.6% respectively. 10μM ZAL inhibited proliferation 33±9.7%; 100μM was lethal. JAr cells were synchronized in G1 using centrifugal elutriation and exposed to 20μM Cd, 20μM ZAL or 20μM Cd + 20μM ZAL for 24 hours. Cd caused cells to remain in G1; cells treated with 20μM ZAL progressed through S phase and into G2. ZAL and Cd together allowed JAr cells to leave G1 and enter S phase, partially relieving the cycle block produced by Cd. First trimester HPE (4–7 mg wet weight) were grown on Matrigel and treated with ZAL (10 or 100μM), Cd (40μM) or ZAL (100μM) plus Cd (40μM) for 6 or 24 hours. Fresh media was then added; explants remained in culture for 6 days. Proliferation was determined by staining sections with proliferating cell nuclear antigen (PCNA) antibody. ZAL (10 and 100μM) did not alter proliferation; Cd significantly inhibited proliferation by 48±5.4%. In ZAL+Cd explants, ZAL protected against the Cd inhibition of proliferation. In summary, Cd inhibits cell proliferation in two human placental cell models. Protection by ZAL demonstrates the importance of calmodulin in mediating the toxicity of Cd in implantation processes. (Supported by ESO2774, ESO1247)

669 NASAL EPITHELIAL CELL PROLIFERATION IN RATS EXPOSED TO VINYL ACETATE VAPOR

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Vinyl acetate (VA) is used in the manufacture of polymers for the paint, adhesives and paper board industries. Chronic inhalation bioassays show that VA causes nasal tumors in rats and olfactory degeneration in rats and mice. Metabolic and inhalation dosimetry studies show that VA is extensively metabolized to acetic acid and acetaldehyde. Acetaldehyde is further oxidized to acetic acid. It is proposed that intracellular accumulation of acetic acid overwhelms cellular buffering capacity and results in cytotoxicity. A weak genotoxicity profile, a long latency to tumor onset, and the cytotoxic effects of VA suggest a strong role for restorative cell proliferation in the mechanism of carcinogenesis. In this study rats were exposed, under bioassay conditions for 1, 5 or 20 exposures to 0, 50, 200, 600, or 1000 ppm vinyl acetate vapor. Histopathological evaluation revealed no effects at 200 ppm and 100% incidence of olfactory degeneration at ≥ 600 ppm. Nasal lesions decreased in severity with concentration and along an anterior-to-posterior gradient. Degeneration and regenerative hyperplasia were observed in respiratory epithelium (1000 ppm only) and olfactory epithelium. Labeling indices were determined by i.p. administration of BrdU and immunohistochemistry. Concentration-dependent increases in respiratory epithelium were observed only after 1 exposure. In olfactory epithelium, labeling indices were elevated after one exposure, returned to control levels after 5 exposures, and rebounded following 20 exposures. These studies suggest a dynamic adaptive response of respiratory epithelium to vinyl acetate exposure and a complicated biphasic response of olfactory epithelium. The results are consistent with a strong role for cell proliferation in vinyl acetate carcinogenesis. Supported in part by the Vinyl Acetate Toxicology Group, Inc.

670 CHEMICAL TRANSFORMATION OF MOUSE HEPATOCYTES DOES NOT INVOLVE DISRUPTION OF CYCLIN-DEPENDENT KINASE PROTEIN COMPLEXES

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Uncontrolled cell growth and proliferation are defining characteristics of neoplasia. Key protein complexes involved in regulating cell division are composed of cyclins, cyclin-dependent kinases (CDKs) and CDK inhibitors (CDIs). Evidence has suggested that disassociation of these proteins is correlated with general cellular transformation in human cells. Therefore studies were initiated to determine whether cell cycle protein complexes were disrupted after chemical transformation of mouse hepatocytes. To characterize cyclin-CDK-CDI complexes in murine liver cells, immunoprecipitation reactions were performed using cellular lysates collected from hepatocytes of 28 day old B6C3F1 mice and spontaneously immortalized hepatocyte cell lines. Complexes were also analyzed in chemically transformed mouse hepatocytes. Cell cycle complexes from B6C3F1 liver cells consisted of a cyclin, a CDK and $p27^{KIP1}$. In the immortalized mouse hepatocytes, complexes were composed of a cyclin, its CDK partner and either $p21^{WAF1}$ or $p27^{KIP1}$. Binary complexes were also detected which included a 14 kDalton protein and either CDK-4 or CDK-6. Further analysis revealed that all cell cycle protein complexes remained intact after chemical transformation. These results suggest that subunit rearrangement is not always associated with cellular transformation as previously reported. Also, differences exist in the types of CDK protein complexes that are expressed *in vitro* or *in vivo*. Finally, these studies have identified key molecules involved in mouse hepatocyte cell cycle control that will allow for further investigation of potential alterations in chemically induced B6C3F1 hepatic tumors.

671 EXPRESSION OF EARLY RESPONSE GENES IN RAT HEPATOCYTES AND LIVER SLICES

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Hepatocyte proliferation is associated with activation of protooncogenes c-myc and c-jun while their differentiation is regulated by transcription factors C/EBPα and C/EBPβ. The pattern of the mRNA expression of these genes was studied in hepatocytes and in precision cut liver slices. Hepatocytes were seeded on collagen coated Permanox dishes and culltured for up to 48 hrs in growth medium (DMEM/F-12 +ITS±EGF) or in differentiation maintenance medium (Chee's + DMSO, Dexamethasone, ITS±EGF) and cultured for 21 days followed by transfer to growth medium. Liver slices, cultured in RPMI + 5% serum, hydrocortisone hemisuccinate, methionine and ITS, were oxygenated and incubated on a dynamic roller system at 37°C. Northern blots revealed that hepatocyte isolation and short-term culture in growth medium induced sustained expression of c-myc and c-jun mRNA. Their expression declined during long-term culture in differentiation maintenance medium but was stimulated upon transfer to growth condtions. C/EBPα expression decreased while C/EBPβ increased in short-term culture. During long-term culture both decreased but transfer to growth conditions stimulated C/EBPβ. In liver slices, c-myc, c-jun and C/EBPβ expression was transiently induced whereas C/EBPα expression remained unchanged. This study indicates that the long-term and liver slice 'in vitro' systems mimic regenerating

liver. Supported by grants AA10154, CA36701 and The Center for Alternatives to Animal Testing.

672 THE ROLE OF HEPATIC CELL DIVISION AND TISSUE REPAIR IN METHANOL POTENTIATION OF CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY

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Methanol (MeOH) has been shown previously to potentiate CCl_4 hepatotoxicity. The objective of the present work was to investigate the role of hepatic cell division and tissue repair in MeOH potentiation of CCl_4 hepatotoxicity. The time-course of injury and recovery after CCl_4 dosing of MeOH pretreated rats was investigated. Male F-344 rats, ~60 days old, were exposed to 0 or to 10,000 ppm MeOH by inhalation for 6 hr and then gavaged with either 0.075 ml CCl_4/kg or corn oil (CO) 18 hr after completion of MeOH exposure. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in serum were measured as indicators of liver damage at 0, 0.75, 1.5, 3, 6, 12, 24, 48, 72, 96 and 120 hr after CCl_4 or CO gavage. MeOH alone had no effect on ALT and AST at any time-point. Both CCl_4 alone and MeOH + CCl_4 increased ALT and AST, beginning at 6 hr for CCl_4 alone and 1.5 hr for MeOH + CCl_4. ALT and AST peaked at 24 hr when MeOH-+CCl_4 was ~50 fold greater than CCl_4 alone. ALT and AST returned to control levels by 120 hr for MeOH + CCl_4 and 48 hr for CCl_4 alone. In another experiment, hepatic cell division and tissue repair were measured by ^{3}H-thymidine (^{3}H-T) incorporation into hepatocellular nuclear DNA at 0, 6, 12, 24, 36, 48, 72, 96, and 120 hr after CCl_4. Compared to the rats receiving CCl_4 alone, ^{3}H-T incorporation was higher in the rats receiving 10,000 ppm MeOH + CCl_4 starting at 24 hr (~6 fold higher), peaking at 36 hr (~9 fold higher) and continuing till 72 hr (~4 fold higher). The present results indicate that inhibition of tissue repair may not play a critical role in MeOH potentiation of CCl_4 toxicity but enhanced tissue repair is consistent with the survival of these rats from marked hepatic injury. (Funded by EPA/UNC-CH TA 901915. This abstract does not necessarily reflect EPA policy.)

673 TISSUE REPAIR: A CRITICAL DETERMINANT OF THE ULTIMATE OUTCOME OF HEPATOTOXIC INJURY

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Earlier literature has reported the importance of tissue repair in determining the ultimate outcome of liver injury. Fischer 344 (F344) rats are reportedly 75-fold more sensitive than Sprague Dawley (SD) rats to 1,2-dichlorobenzene (o-DCB) hepatotoxicity. No information is available on whether these differences in liver injury are reflected in differences in animal mortality. LD50s in male rats were not different (1.66 ml/kg for F344 and 1.76 ml/kg for SD rats). Therefore, the objective of this study was to investigate the role of tissue repair in the observed equalization of ultimate toxicity in the two strains. A dose-response (0.2, 0.6 and 1.2 ml o-DCB/kg) study was performed in which injury and tissue repair occurring as two dynamic but opposing events were measured over a time course (0–96 hr) in age-matched male F344 and SD rats. Higher palsma ALT elevations were observed in F344 rats around 36-48 hr. Liver regeneration was assessed by measuring ^{3}H-thymidine (^{3}H-T) incorporation into hepatonuclear DNA. Increased incorporation of ^{3}H-T was seen in F344 rats around 36–48 hr signifying an increase in S-phase synthesis. The importance of tissue repair in deciding the ultimate outcome of liver injury was investigated by colchicine antimitosis. Blocking the tissue repair response with colchicine in F344 rats resulted in forty % lethality in F344 rats. The findings suggest a critical role of tissue repair in overcoming the significantly higher liver injury in F344 rats to equalize the ultimate outcome of liver toxicity to match that observed in SD rats. (Partly supported by a grant from ATSDR).

674 EFFECT OF UDP-GLUCURONOSYLTRANSFERASE INDUCERS ON THYROID CELL HYPERPLASIA

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UDP-GT inducers, which increase thyroid stimulating hormone (TSH), increase the proliferation of thyroid follicular cells. It has been proposed that increases in the labeling index (LI) results in even greater increases in the total number of dividing cells. Therefore, we hypothesized that those UDP-GT inducers which increase serum TSH and thyroid follicular cell proliferation would increase the total number of follicular cell divisions (TNFCD), and lead to large increases in the total number of follicular cells (TNFC). Rats were fed diets containing various doses phenobarbital (PB), pregnenolone-16α-carbonitrile (PCN), 3-methylcholanthrene (3MC) or Aroclor 1254 (PCB) for 7 days. TSH was quantified by RIA. Thyroid follicular cell proliferation was quantified by PCNA immunohisto-chemistry and expressed as a LI. TNFC was determined using computerized image analysis. TNFCD was determined by multiplying the LI and TNFC. Serum TSH was increased by PB (60%) and PCN (90%). The LI was also increased by PB (300%) and PCN (600%). TNFCD was increased dramatically by PB (600%) and PCN (1,300%). However, TNFC was increased only slightly by PCN (43%). In conclusion, increases in thyroid follicular cell proliferation resulted in even greater increases in the total number of dividing cells, but did not lead to the expected marked increase in the total number of follicular cells. (Supported by NIH Grant ES-07079).

675 ROLE OF HEPATIC PROSTAGLANDINS IN THE INDUCTION OF DNA SYNTHESIS BY PHENOBARBITAL AND THE PEROXISOME PROLIFERATOR CIPROFIBRATE IN RATS

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Peroxisome proliferators (PP) are a class of chemicals that induce and promote hepatic tumors in rodents. These compounds are not genotoxic, and the mechanism by which they induce tumors is not fully understood. Phenobarbital (PB) also is a hepatic tumor promoter that produces a different natural history during the promotion of hepatocarcinogenesis. In addition, opposite effects on hepatic eicosanoid concentrations have been demonstrated previously. In this experiment, we examine whether higher hepatic eicosanoid concentrations are necessary for the induction of DNA synthesis after the administration of PB or the PP ciprofibrate (CIP). PB (0.05% in diet) or CIP (0.01% in diet) was fed to rats from 1 to 10 days. For the rats treated with CIP, the peroxisomal enzyme fatty acyl-CoA oxidase increased gradually from day 1 to day 10. PB treated rats had a higher cytochrome P450 2B1/2 activity over the entire course of feeding. Hepatic prostaglandins E_2 and $F_{2\alpha}$ concentrations were significantly reduced in the rats treated with CIP, while no significant differences were seen between the control and PB-treated rats. DNA synthesis was increased in both PB-treated and CIP-treated rats. These results show that higher eicosanoid concentrations are not necessary for induction of DNA synthesis by CIP or PB. (Supported by NIH grants CA-43719 and CA-01688)

676 REGIONAL CHANGES IN CELL PROLIFERATION IN THE RAT NOSE AFTER INHALATION EXPOSURE TO HMPA

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Hexamethylphosphoramide (HMPA) is a rat nasal carcinogen that induces squamous cell carcinomas in the anterior portions of the nasal cavity following chronic inhalation exposures as low as 50 ppb. These tumors may arise as a result of P-450-mediated release of formaldehyde (HCHO) another nasal carcinogen. Regional induction of cell proliferation (CP) was measured by BrdU incorporation and PCNA expression. Rats exposed nose-only to 3 ppm HMPA for 6 h, and sacrificed 18, 48, 96 or 144 h post-exposure, showed statistically significant increases in CP. Histological evaluation revealed a slight increase in PAS staining along the septum at all time points, but no other remarkable changes. CP was assessed in sections of nasal tissue described by Mery *et. al. (Tox. Pathol.* **22**(4):353). At 18 h post-exposure there was an increase in CP in squamous epithelium, which returned to control levels by 96 h. CP in section 6 increased significantly by 96 h. Along the dorsal meatus in section 9, olfactory basal cell labeling initially decreased then returned to control levels by 96 hours. The labeling index in the dorsal meatus of non-basal cells (primarily sustentacular) increased with time and reached a maximum at 96 hours. PCNA expression was consistent with BrdU trends. Monticello *et al.* (TAP **111**:409) reported HCHO-induced tumors in the rat nose at 6 ppm, but not 2 ppm. These tumors correlated with cytotoxicity-associated CP. HMPA induces CP and tumors, but not cytotoxicity, at approximately 3 ppm. These data suggest that HMPA induces proliferative, perhaps mitogenic, responses in the nasal epithelium and this response may facillitate the fixation of low level genetic damage induced by liberated HCHO.

677 EXPOSURE OF BISPHENOL A, AN ENVIRONMENTAL ESTROGEN, PRODUCES ADVERSE EFFECTS IN THE MAMMARY GLAND

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Bisphenol A is an environmental estrogen-like chemical shown to mimic the actions of estrogen both in vivo and in vitro. In the present study we examined the effects of bisphenol A, on the proliferative activity, cell cycle kinetics and the differentiation of the mammary gland of female Noble rats. The exposure of animals to 5.4 mg/day of bisphenol A significantly increased proliferation of the mammary epithelial cells in all structures, whereas, exposure to 0.01 mg/day had appreciable effect only in the TEBs. Both labeling index and growth fraction were increased by bisphenol A treatment. Labeling index and growth fraction were 19% and 27%, respectively, for a low dose of bisphenol A and 27% and 45%, respectively, for a high dose of bisphenol A, compared to 18% and 35%, respectively, of controls. In addition, bisphenol A exposure altered cell cycle kinetics. A significant increase in the conversion of mammary epithelial cells from G0 to G1 and S-phase cells by 1.8 and 4.5-fold, respectively, was observed in the high dose group compared. The low dose group of animals showed a reduction in the conversion of mammary epithelial cells from G0 to G1-phase cells by 0.5-fold but an increase in S phase cells by 1.9-fold compared controls. Differentiation measured by the degree of lobular maturation revealed that the conversion of immature structures to mature structures was significantly increased in response to high dose of bisphenol A exposure compared to that of control. Low dose of bisphenol A did not show any appreciable increase in lobular maturation. Perturbation of cell cycle in response to bisphenol-A exposure may produce adverse effects in the mammary glands of the Noble rats.

678 MELATONIN ATTENUATES PROLIFERATION IN MCF7, BG-1 AND HELA CELLS

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Electromagic fields (EMF) reduce both the quantity of melatonin produced by the pineal gland, and its effectiveness at the target site. Melatonin is a indolic pineal hormone important in controlling biological rhythms and produced primarily at night in mammals However, recent evidence suggests that melatonin has high affinity binding sites, possibly receptors, which are present in many peripheral organs, in addition to the brain. Previous research has demonstrated that melatonin can attenuate proliferation in estrogen responsive tissues. We are interested in examining whether melatonin effects growth factor induced proliferation, and if melatonin can attenuate proliferation in estrogen receptor negative cells. MCF7, BG-1 (ER+) and HeLa cell (ER-) were treated with melatonin 24 hours prior to growth factor stimulus (10ng/mL EGF or IGF-1). Cells were allowed to proliferate for five days, and then assayed for growth colorimetrically (MTT) on day six. EGF induced proliferation was attenuated at pharmacological concentrations of melatonin (10^{-5}M) in MCF7 cells, however melatonin did not attenuate EGF induced growth in BG-1 or HeLa cells. In contrast, IGF-1 induced proliferation was attenuated in BG-1 and HeLa cells by melatonin at physiological and pharmacological concentrations (10^{-9} to 10^{-5}M), however melatonin did not attenuate proliferation in MCF7 cells stimulated with IGF-1. These experiments demonstrate melatonin attenuates growth factor stimulated proliferation, and that melatonin has different responses towards growth factors in distinct cells lines. Furthermore, melatonin can attenuate proliferation in an estrogen receptor negative cell line. Lastly, preliminary data suggests that melatonin exposure may reduce IGF-1 stimulation of the signal transduction cascade in BG-1 cells.

679 ANTIOXIDANTS SELECTIVELY SUPPRESS STEM CELL FACTOR (SCF) SYNERGY IN MURINE HEMATOPOIETIC PROGENITOR CELLS (HPC)

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Reactive oxygen species (ROS) stimulate proliferation and growth responses in many mammalian cells. We examined the effects of ROS and various antioxidants on cytokine response in murine HPC. Pretreatment of C57BL/6 HPC with xanthine + xanthine oxidase (X/XO) produced a dose-dependent enhancement of clonogenic response to granulocyte-macrophage colony stimulating factor (GM-CSF). Normal enhancement of GM-CSF by SCF was not additive with X/XO indicating the same subpopulation of HPC was targeted in each case. Mutant mice strains that lack a SCF responsive population of HPC (W/W^v and Sl/Sl^d) are resistant to X/XO and SCF induced enhancement of GM-CSF. Additionally, 3,4 epoxybutene, which selectively abrogates SCF synergy with GM-CSF also inhibited X/XO enhancement. These findings suggest that ROS might play a role in normal SCF mediated proliferation of HPC. Therefore, we tested the antioxidants N-tert-butyl-α-phenylnitrone (PBN), superoxide dismutase (SOD) and catalase. PBN and SOD effectively inhibited both SCF and X/XO synergism with GM-CSF while catalase had no effect suggesting that the superoxide anion may be involved. Antioxidant sensitivity appears to be population-specific as SCF synergy with IL-3 or G-CSF were not effected. These findings indicate that X/XO and SCF stimulate a common subpopulation of murine HPC and that SCF synergy with GM-CSF is sensitive to antioxidant inhibition.

680 NUTRITIONAL MODULATION OF STIMULATED CELL PROLIFERATION IN A HUMAN HEPATOMA CELL LINE

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Cell proliferation and tissue repair occur as a compensatory response to injury and play a decisive role in the outcome of toxicity. Previous studies have shown that 15% glucose in drinking water for 7 days can inhibit this compensatory cell proliferation interfering with tissue repair in the liver. These findings raise questions concerning the value of using glucose infusions as a source of energy in emergency medicine. Hence, this study was conducted to develop an *in vitro* model to study the molecular mechanism(s) of inhibition of cell proliferation using human hepatoma (HuH7) cells. Doubling time was 36 ± 2 hr. Dose-response studies conducted with 12-fold dose-range of TA (0–300 ppm) indicate a dose-dependent increase in cell numbers upto 150 ppm with 100% cytotoxicity observed at 300 ppm. Also, dose-response studies conducted with a 5-fold dose range of glucose (0.2–1%) indicate that while 0.2% (control) and 0.5% glucose do not interfere with cell growth, 0.75 and 1% suppress cell division. Further, when cells were grown in the presence of glucose for 7-days (mimicking *in vivo* studies) and challenged with TA on day 8, the lowest concentration of glucose (0.5%) significantly diminished cell proliferation induced by 150 ppm TA. Currently, studies are underway to examine a detailed time-course (0–96 hr) of the biochemical effects of thioacetamide on stimulation of cell proliferation and glucose-induced inhibition of this stimulated cell proliferation. Following the biochemical studies, alterations in proto-oncogene expressions will be examined to understand the molecular mechanisms of glucose-induced alterations cell proliferation by glucose. This study will help in establishing an *in vitro* model to study hepatotoxicity and tissue repair and also provide a model to investigate the molecular events and mechanisms of inhibition of cell proliferation by nominal concentrations of glucose. Findings from such studies will help us to understand mechanisms of altering cell proliferation processes by nutritional modulation and in the development of strategies to treat acute liver injury.

681 α-NAPHTHYLTHIOUREA AUTOPROTECTION

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In the 1940s α-naphthylthiourea (ANTU) autoprotection was discovered in wild Norwegian rats exposed to ANTU poison baits. Autoprotection was thought to be due to a decrease in Cyp P-450 resulting from the initial exposure. However, studies have shown that bioactivation of radiolabeled ANTU is not decreased in the autoprotected rats. To test our hypothesis that stimulated cell division and tissue repair underlie ANTU autoprotection, we developed autoprotection and heteroprotection models. For ANTU autoprotection, 5 mg/kg, po was given to male Sprague-Dawley rats, followed by 70 mg/kg, po 24 h later. For heteroprotection by ANTU, paraquat (PQ) which undergoes redox-cycling to inflict free-radical injury in the lung was chosen. Rats were treated with a low dose of ANTU (5 mg/kg, po) 24 h before challenge with a 100% lethal dose (180 mg/kg, po) of PQ. This protocol resulted in a 80% protection against the lethal effect of PQ. PQ autoprotection model was also developed (25 mg/kg, po; 72 h later, 180 mg/kg, po). ANTU and PQ autoprotection lasted until 10 days, all protection being lost on day 20. [^{3}H]-T incorporation studies in the ANTU autoprotection model indicate a stimulation of S-phase synthesis in the lung tissue after being challenged with the lethal dose of ANTU. From pulse-labelling experiments, tracheobroncheolar and parenchymal tissues were surgically separated and ^{3}H-T was

estimated in the ANTU autoprotection model. ^{3}H-T incorporation primarily occurs in the alveolar region. These studies suggest the possibility that pulmonary cell division and tissue repair induced by ANTU facilitate sustained pulmonary tissue repair after subsequent ANTU-induced lung injury, leading to recovery from lung injury, and protection against ANTU lethality.

682 COMPARISON OF KI-67 AND PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) EXPRESSION IN UTERINE LEIOMYOMAS (LM)

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Uterine LM "fibroids" are the most common benign tumors found in US women over 30 years of age. Clinically, these tumors expand in size and may increase up to several centimeters in diameter; however, the proliferative rates of uterine LM are reported to be low. PCNA and Ki-67 are proliferation markers that are commonly used in immunohistochemistry (IHC). PCNA is an intranuclear polypeptide that is present in replicating cells during the late G1 and S phases of the cell cycle; whereas, Ki-67 is a non-histone nuclear protein found in replicating cells in the S phase, as well as, the G1, G2, and M phases of the cell cycle. In this study, we assessed the expression of PCNA and Ki-67 in formalin-fixed, paraffin-embedded sections of normal myometrium and uterine LM taken from 8 women. PCNA and Ki-67 labeling indices (LI) were calculated and compared for these tissues. The results of this study showed that 67% (4/6) of the normal myometrium samples were positive for PCNA and 50% (3/6) were positive for Ki-67; whereas, 84% (41/49) of the tumor samples were positive for PCNA, and only 22% (11/49) were positive for Ki-67. Mean Ki-67 LI for normal myometrium and tumors were 0.15% and 0.09%, respectively. Using PCNA, the mean LI for normal myometrium and tumors were 0.23% and 0.99%, respectively. These data suggest that PCNA and Ki-67 IHC give similar result for normal myometrium; however, the PCNA method produced consistently higher labeling values in the LM than Ki-67. A marginally significant difference ($p<0.05$) was present between mean LI of normal and tumor sections using PCNA IHC; however, this response was not observed in the Ki-67 stained sections. These data suggest that PCNA staining is the method of choice for detecting the proliferative activity of cells in formalin-fixed paraffin-embedded samples of normal myometrium and uterine LM.

683 EFFECT OF HEXACHLOROBENZENE (HCB) ON ESTROGEN-DEPENDENT CELL PROLIFERATION IN HUMAN MAMMARY EPITHELIAL CELLS

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Organochlorines, such as HCB, are persistent and widely distributed contaminants in the environment. Recently, organochlorine pesticides have been linked to human breast cancer: Milk, blood and fat tissues from women with breast cancer contained higher amounts of HCB, DDE or β-HCH than those from healthy women. However, these compounds do not possess any mutagenic potential. HCB is also known to induce liver tumors primarily in the female rat. This study aimed to establish if in the human breast HCB acts through an epigenetic pathway involving induction of proliferation. Human mammary epithelial cells, MCF-10A, were incubated (48 h) with various concentrations of HCB (10 – 100 μM) in the presence or absence of 1 nM estradiol. No cytotoxicity was observed as measured by neutral red uptake. Cell proliferation, as measured by incorporation of BrdU (last 6 h of treatment) into DNA, was induced only by the HCB/estradiol combination. The response increased with higher doses of HCB. This indicates the importance of concomitant exposure for mitogenicity. This activity observed *in vitro* may be related to a higher risk of human breast cancer in women exposed to organochlorines. (Supported by MRCC)

684 CHLOROFORM-INDUCED CYTOTOXICITY IN BDF$_1$ MICE

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Chloroform has been reported to induce kidney tumors in male but not female BDF$_1$ mice; liver tumors were not increased in either sex. Since tumor induction appears to be through a nongenotoxic/cytotoxic mode of action, the toxicity in BDF$_1$ mice was investigated. Male and female mice were exposed to chloroform vapor concentrations of 0, 0.3, 5, 30, or 90 ppm 6 hr/d for 4 d. Bromodeoxyuridine (BrdU) was administered via osmotic pumps implanted 3.5 d prior to necropsy. The labeling index, % of cells in S-phase, was quantified using BrdU immunohistochemistry. Subgroups of male mice were exposed 5 d/wk for 2 wk to 30 or 90 ppm. Concentration-dependent cytotoxicity in the proximal tubules (PT) of the cortex of male mice ranged from mild vacuolation to severe degeneration. Histological evidence of regeneration in the PT epithelial cells was present. The kidneys were minimally affected in female mice. Hepatocyte vacuolation, degeneration, and regenerative proliferation were present in male and female mice exposed to 30 or 90 ppm. In male mice exposed for 2 wk, 2/5 and 4/5 mice exposed to 30 or 90 ppm, respectively, died with severe toxicity in the kidney. The present results are consistent with the hypothesis that chloroform-induced tumors are dependent on a cytotoxic and regenerative response in the target organ. However, increases in kidney tumors were reported only in male mice exposed to 30 or 90 ppm. To achieve these exposures, mice were conditioned by sequentially increasing the chloroform concentration over a period of weeks. Survival data from the present study indicate that 30 and 90 ppm exceed a MTD and the acute mortality would be unacceptable in a long-term study without a step-up period.

685 OXIDANT-INDUCED CELLULAR SENESCENCE

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Acute exposure of late-passage normal human keratinocytes to high concentrations of tert-butyl hydroperoxide (tBHP; 10–100 uM for 8 hrs.) inhibited mitogen-induced DNA synthesis in a concentration-dependent manner. Moreover, treatment with 100 uM tBHP resulted in a permanent arrest of growth factor-induced cell proliferation without significant cytotoxicity, as determined by LDH release and cell enumeration techniques. Propidium iodide staining of DNA content demonstrated no differences in the distribution of cell populations immediately following tBHP treatment; however, within 24 hours following the cessation of oxidative insult, tBHP treatments of 10, 40 and 70 uM had resulted in the appearance of a significant subpopulation (40–45%) of "hypodiploid" cells; by contrast, 100 uM tBHP exposure had increased the percentage of "senescence-like" cells to greater than 70%. While tBHP-arrested keratinocytes failed to respond mitogenically to the exogenous factors routinely used to promote cell proliferation, the same arrested cell population did respond to endogenous factors present in keratinocyte-conditioned growth medium. Media collected from cultures of rapidly dividing human keratinocytes had previously been demonstrated to contain an endogenous factor capable of promoting immediate cell cycle progression into the DNA synthesis phase, independent of exogenous factor stimulation. In contrast to results from acute tBHP studies, chronic exposure of the same late-passage human keratinocytes to either tBHP (1–10 uM, 5–7 days) or diamide (1–10 uM, 5–7 days), a glutathione-depletion agent, significantly increased the level of DNA replication as compared to non-treatment controls.

686 ALTERATIONS IN CELL SIGNALING BY TRICHLOROACETATE

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Trichloroacetate (TCA) is a peroxisome proliferator (PP) and hepatocarcinogen in rodents. The mechanism of carcinogenicity of PPs is unknown, but may be due to enhancement of cell proliferation and/or suppression of apoptosis in initiated cells. To determine if TCA alters the expression of proteins involved in controlling cell proliferation, we examined the expression of cyclin dependent kinase CDK1, and the mitogen-activated protein kinases (Erk1/2) in normal and tumor tissue of male B6C3F1 mice. Mice were treated with 2.0 g/L TCA in their drinking water for 50 weeks, then were switched to 0, 0.02, 0.5, 1, or 2 g/L TCA for an additional two weeks. CDK1 levels were 3 to 10-fold higher in tumor than normal tissue. CDK1 levels were also more than 2-fold higher in normal liver homogenates from animals treated with 2 g/L TCA for 52 weeks compared to animals from which TCA was removed for the final two weeks. The presence of 2 g/L TCA slightly suppressed ERK kinase levels in normal liver and this effect was greatly enhanced in tumor tissue. The results indicate that TCA alters cell signaling pathways controlling cell division, which may contribute to TCA-induced carcinogenesis. Supported by NIEHS RO1-ES04648 and Laboratory Directed Research and Development under U.S. D.O.E. contract DE-AC06-76RLO 1830.

687 VALIDATION AND USE OF IMMUNOCYTOCHEMICAL MARKERS OF CYTOCHROME P450 INDUCTION AND CELLULAR PROLIFERATION IN RODENT LIVER

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Cytochrome P450 induction and cellular proliferation are important pathological indicators of toxicity. Mixed function oxidase induction may result in metabolism of xenobiotics to reactive metabolities, whilst increased cell turnover may increase the potential for DNA damage. Such indicators are important aspects of risk assessment and can serve to give an early window into toxic changes. This study set out to evaluate morphological markers of cytochrome P450 induction and cellular proliferation in a system known to produce such changes. Ten five week old mice were killed 3 days after induction with 40mg/kg phenobarbitone and 100mg/kg β-napthoflavone. The livers were fixed immediately after death for 24 hours in neutral buffered formaldehyde, and then processed for immunocytochemical evaluation with anti human P450 2C10 and PCNA (proliferating cell nuclear antigen). Hepatocellular hypertrophy accompanied by positive p450 and PCNA staining were apparent, illustrating the validity of the markers and their cross reactivity. Livers from spontaneous lesions (hypertrophy, cellular atypia and adenomas) arising in a mouse and rat carcinogenicity study were evaluated in a similar way. Enzyme induction and cellular proliferation were demonstrated indicating that these changes may occur as early and late events.

688 THE IPCS COLLABORATIVE STUDY ON NEUROBEHAVIORAL SCREENING METHODS

V C Moser. *Neurotoxicology Division, NHEERL, US EPA, RTP, NC*

The International Programme on Chemical Safety (IPCS) sponsored a collaborative study to evaluate the utility of neurobehavioral test methods for identifying neurotoxic chemicals. The goal was to determine the intra- and interlaboratory reliability of a functional observational battery (FOB) and an automated assessment of motor activity. These test methods are currently recommended to screen chemicals for neurotoxic potential in the U.S., and these results are timely in light of ongoing efforts to harmonize testing guidelines worldwide. This study included four laboratories in the U.S. and four in Europe, each of which evaluated the effects (dose-response and time-course) of seven prototypic chemicals following both acute and 4-week exposures. These data provide important information regarding the reliability, sensitivity, and robustness of neurobehavioral screening methods over a range of laboratory conditions. The results of the chemical tests indicated that all participating laboratories generally could detect and characterize the effects of known neurotoxicants, in spite of differences on specific endpoints. Control data were quite similar for some endpoints, but varied greatly for others. This workshop will 1) provide the background and study design of this collaborative effort; 2) focus on the presentation of the data, concordance of the results across laboratories; and 3) provide opportunity for discussion on issues raised regarding the conduct and interpretation of neurotoxicity screening studies.

689 ROUNDTABLE: PROPOSITION 65 - WHAT'S HAPPENING TO THE SCIENCE?

J N Boyd. *Corporate Safety and Health, Corning Incorporated, Corning, NY*

The California Safe Drinking Water and Toxics Enforcement Act of 1986, commonly referred to as Proposition 65, states: "No person in the course of doing business shall knowingly and intentionally expose any individual to a chemical known to the state to cause cancer or reproductive toxicity without first giving clear and reasonable warning to such individual." Either the state or private citizens may bring suit against those businesses whose processes or products allegedly result in exposures in excess of "no significant risk levels" (NSRLs) for carcinogens or 1/1000 of the NOEL for reproductive toxicants. In this the tenth year since the law was enacted, a number of legal and technical issues remain unresolved. Of particular interest to toxicologists are: (1) criteria for establishing NSRLs and NOELs and (2) exposure assessment methodology and assumptions. These are critical components in determining compliance or non-compliance for a product or process. Presentors will include attorneys involved in both prosecuting and defending Proposition 65 cases and toxicologists involved in resolution of the underlying scientific issues. They will discuss: 1) use of tabulated Tumorigenic Dose 50 (TD50) values in an expedited method for estimating carcinogen potency and NSRLs 2) the regulatory advantages of the expedited method 3) uncertainity and ambiguity in the application of exposure assessment criteria in establishing compliance for specific products 4) the role of scientific and non-scienbtific criteria in enforcement.

690 AQUATIC POLLUTION-INDUCED IMMUNOTOXICITY IN WILDLIFE SPECIES: INTRODUCTION

R W Luebke. *U.S. EPA, Research Triangle Park, NC*

At present, immunotoxicity studies are, with few exceptions, conducted under laboratory conditions and nearly all of these studies are done in laboratory rodents. As such, many toxicologists are only vaguely aware of the immunotoxicity studies done under the more natural condition of environmental exposure to pollutants. The purpose of this symposium is threefold: the dissemination of information on the immunologic health of selected wildlife species in contaminated habitats; to demonstrate the plausibility of using altered immune responses of directly-exposed species as a biomarker to predict the effects of toxic environmental pollutants; and to foster collaboration between field and laboratory immunotoxicologists. The first speaker is Peter Hodson, president of SETAC. He will address trends in aquatic pollution and provide specific examples based on pulp mill effluents. The next three speakers will discuss the effects of polluted environments on feral populations of fish (Mohamed Faisal), birds (Keith Grasman) and seals (Peter Ross) living in these contaminated environments. The final speaker (Judy Zelikoff) will discuss how well laboratory immunotoxicological studies in wildlife species compare with data generated in field studies. All of the speakers in this symposium are actively involved in evaluating immune function in wildlife species under field or laboratory conditions and are uniquely suited to discuss the ecological, immunological and logistical aspects of these studies.

691 TRENDS IN WATER POLLUTION EFFECTS ON FISH

P V Hodson. *National Water Research Institute, Environment Canada, Burlington, ON, C M Couillard, Maurice Lamontagne Institute, Fisheries and Oceans, Mont-Joli, PQ.* Sponsor: R. W. Luebke

Pulp mill effluents provide excellent examples of trends in pollution effects on fish. Because fish kills are increasingly rare, sublethal impacts have become evident as fish re-colonize receiving waters and our ability to recognize and measure effects improves. The exposure of fish to compounds such as chlorinated phenolics, resin and fatty acids, and chlorinated dioxins and furans is associated with induction of hepatic MFO enzymes, altered stress responses, possible immune impairment, pathology, changes in parasitic infection, genotoxicity, increased growth rates, altered energy metabolism, impaired sexual maturation, and recruitment failure. These effects can be detected at distances of up to 100 km from the source. The result is a change in fish population demographics as the balance shifts among growth, mortality and reproduction. Despite an absence of acute lethality, sublethal impacts on single species impair sports and commercial fisheries, and can change ecosystem structure and function. Some of the diseases associated with exposure to pulp mill effluents, including increased rates of parasite infection, proliferation of macrophage aggregates, and tumors, may be a consequence of immunotoxicity. Research is needed to develop and validate markers of impaired immune responses in fish in laboratory, mesocosm, and epidemiological studies to demonstrate whether pulp mill effluent affect the incidence of diseases in fish.

692 POLLUTION-INDUCED IMMUNOTOXICOLOGICAL ALTERATIONS IN FERAL FISH POPULATIONS

M Faisal. *Virginia Institute of Marine Science, The College of William and Mary, Gloucester point, VA 23062.* Sponsor: R W Luebke

Chemically contaminated estuarine sediments may affect many physiological systems of aquatic organisms. For example, fish collected from sites in the Elizabeth River in Virginia, whose sediments are heavily contaminated with polycyclic aromatic hydrocarbons (PAH), exhibit a high incidence of cancers. Our studies have shown that the cell-mediated immune responses of cancer-bearing fish are severely suppressed. Moreover, it was shown that lymphocytes of two fish species are capable of activating high molecular weight PAH into their potentially carcinogenic metabolites and possess high affinity

Ah receptors. Studying the molecular aspects of this immunomodulation has shown that exposure to PAH causes a sharp increase in the levels of cAMP in circulating and splenic leukocytes, followed by an augmentation of DNA synthesis. Moreover, it was possible to demonstrate that covalent PAH (benzo-[a]pyrene)-DNA adducts occur in intoxicated fish. Among the lymphoid organs in fish, the thymus showed the highest number of adducts. Current investigations using PCR technology have shown that exposure of fish to PAH is associated with the expression of three distinct proteins in lymphocytes. The role these induced proteins may play in the observed immunosuppression is being evaluated.

693 IMMUNOLOGICAL BIOMARKERS AND ENVIRONMENTAL CONTAMINANTS IN FISH-EATING BIRDS OF THE GREAT LAKES

K A Grasman. *Department of Biological Sciences, Wright State University, Dayton, OH; P F Scanlon, Department of Fisheries and Wildlife Sciences, VPI & SU, Blacksburg, VA; G A Fox, Canadian Wildlife Service, Hull, Quebec, Canada.* Sponsor: R.W. Luebke

Numerous studies have shown that organochlorine pollutants are associated with developmental and population-level problems in fish-eating birds of the Great Lakes. Because the immune system is sensitive to organochlorines in laboratory animals, this study investigated the effects of pollutants on immune function in Great Lakes birds and evaluated immunological tests as biomarkers for contaminant effects in wild birds. During 1991-94, specific immune functions and general hematological variables were measured in herring gulls (*Larus argentatus*) and Caspian terns (*Sterna caspia*). Study sites were chosen across a wide range of organochlorine contamination caused primarily by polychlorinated biphenyls (PCBs). Several white blood cell variables were associated with contaminants. In herring gull adults, the heterophil to lymphocyte ratio decreased as liver ethoxyresorufin-O-deethylase (EROD), an index of contaminant exposure, increased. In herring gull chicks, thymus mass decreased as EROD increased. T-cell-mediated immunity was measured using the phytohemagglutinin skin test. In gull and tern chicks, T-cell-mediated immunity decreased strongly as egg PCB concentrations increased. The thymic atrophy and T cell suppression observed in this field study were consistent with the reported effects of PCBs and dioxin (TCDD) in laboratory animals. Chicks of both species exhibited biologically significant differences in antibody production among sites, but relationships to contaminants or other factors remain unclear. In gull and tern chicks, plasma retinol (vitamin A) concentrations decreased as egg PCB concentrations increased. Immunological variables are useful for assessing health effects, including those associated with contaminants, in wild birds.

694 CONTAMINANT-RELATED IMMUNOSUPPRESSION IN HARBOR SEALS FED HERRING FROM THE BALTIC SEA

P S Ross, R L de Swart, H H Timmerman, P J H Reijnders, H van Loveren, J G Vos and A D M E Osterhaus. *Seal Rehabilitation and ResearchCentre, Pieterburen; National Institute of Public Health and the Environment; DLO Institute for Nature and Forestry Research; Erasmus University Rotterdam: The Netherlands.* Sponsor: B. Luebke

Mass mortalities among marine mammal populations in recent years have been largely attributed to morbillivirus infections, but contaminant-induced immunosuppression could neither be implicated nor ruled out as a contributing factor. In an immunotoxicological study, we fed herring from the highly contaminated Baltic Sea or the relatively uncontaminated Atlantic Ocean to two groups of 11 captive harbour seals each for 21/2 years. During this time, natural killer cell activity and T-lymphocyte responses, *in vitro*, became impaired in the Baltic group. In addition, specific delayed-type hypersensitivity and antibody responses to ovalbumin were impaired, suggesting systemic implications for the *in vitro* observations. In a follow-up study aimed at expanding on our observations in seals, pregnant rats were given an oil extracted from the same two herring batches on a daily basis, and immune function in their female offspring was examined. Immune dysfunction was evidenced by lower thymus weights and T-lymphocyte responses in thymus and spleen suspensions in the rat pups exposed transplacentally and via mother's milk to the lipophilic contaminants in the Baltic Sea herring. An analysis of the chemical residues in the herring suggested that PCBs contributed the most to the total 2,3,7,8-TCDD toxic equivalents. Since immunotoxicity has been shown to be largely mediated via the *Ah*-receptor, PCBs may pose the greatest immunotoxic threat to free-ranging marine mammals. We conclude that environmental contaminants accumulated in the aquatic food chain may have contributed to the severity and extent of recent virus-induced mass mortalities among marine mammals inhabiting polluted coastal waters of Europe and North America.

695 HOW CLOSE DO LABORATORY IMMUNOTOXICOLOGY STUDIES COME TO PREDICTING POLLUTANT-INDUCED EFFECTS IN FERAL POPULATIONS?

J T Zelikoff. *New York University School of Medicine, Institute of Environmental Medicine, NY, NY*

Through the efforts of a number of different laboratories, a battery of immunological assays is available to predict the immunotoxicity of different classes of xenobiotics. These assays, originally developed in rodents, have been adapted for use in a variety of animals species and are now used routinely in these models to assess the immunotoxicity of different chemical compounds. For example our laboratory has employed a number of these immune assays to assess metal-induced immunotoxicity in laboratory-reared fish. Results of the aforementioned laboratory investigations demonstrate the usefulness of these endpoints to reliably predict immunotoxicity in a teleost system. In studies preformed in the field, Weeks et al. have demonstrated that two immune functional assays commonly used to predict the effects of chemicals on macrophage activity, i.e., phagocytosis and chemotaxis, can be employed successfully to demonstrate the immunotoxic effects of polycyclic aromatic hydrocarbon (PAH)-contaminated aquatic environments on feral fish. The same battery of immunological assays have also been used to assess pollution-associated immunomodulation in other feral species including fish-eating birds, harbor seals, and Beluga whales. This presentation will discuss the results of immunotoxicology studies performed in the laboratory on wildlife species in order to demonstrate the usefulness of these assay systems to predict immunomodulating effects of xenobiotics in "real world" situations. These laboratory studies will then be related to those previously discussed in the program so as to determine the applicability of immunotoxicology assays to be used in field settings. Supported by U.S. Army Medical Research and Materiel Command, Contract No. DAMD-17-93-C-3059.

696 RECOMBINATION AND GENOME REARRANGEMENTS: INVOLVEMENT IN CARCINOGENESIS AND GENOTOXIC ENDPOINTS

R H Schiestl. *Department of Molecular and Cellular Toxicology, Harvard School of Public Health, Boston, MA*

To predict a potential carcinogenic activity of chemicals from genetic toxicology assays it seems important to use a genetic endpoint that is involved in carcinogenesis. In addition to point mutations. DNA recombination and genome rearrangements causing deletions, gene amplifications and translocations are involved in carcinogenesis and in most genetic diseases. Tumor suppressor genes can be removed by deletion events, proto-oncogenes can be turned on after deletion events join them to expressed loci and loss of heterozygosity may uncover the phenotype of recessive mutations. Mutations causing a high frequency of carcinogenesis such as p53, ataxia telangiectasia. Werners syndrome and Blooms syndrome increase genetic instability causing a high frequency of recombination and genome rearrangements. Assays for recombination exist in yeast and in Drosophila. Assays in both organisms have been validated to some extent and they identify recombinagens. New assays have been developed in human cells and in vivo in mice. Deletion events in yeast, in human cells and in the mouse are inducible by carcinogens including carcinogens that are negative in the Salmonella (Ames) assay and in most other short term tests. This symposium will feature presentations on the effects of mutations causing a high frequency of cancer on recombination and genome rearrangements and the molecular characterization of these events. The second part of the symposium will feature an overview of assays detecting such recombination events, their validation status and advantages of their use in genetic toxicology.

697 GENETIC REGULATION OF GENOMIC FLUIDITY IN NORMAL AND NEOPLASTIC CELLS

T D Tlsty. *Department of Pathology, University of California at San Francisco. CA*

Genomic integrity is maintained by a network of cellular activities that assesses the status of the genome at a given point in time and provides signals

to proceed with or halt cell cycle progression. Mutations in any part of these cellular pathways can have the ultimate effect of disrupting chromosomal integrity. We have used viral proteins involved in malignant transformation to investigate cellular pathways that may be perturbed during loss of genomic instability. Recent studies have identified cellular proteins which are targets for the viral oncoproteins, stressing the importance of these cellular proteins in controlling neoplasia. Among the targets of the viral oncoproteins are the products of p53 and retinoblastoma (Rb) tumor suppressor genes. We demonstrate that the expression of human papillomavirus type 16 E6 and E7 oncoproteins in normal, mortal cells disrupts the integration of the network of signals that maintain genomic integrity. E6-expressing cells, in which cellular p53 protein is bound and degraded exhibited alterations in cell cycle control and displayed the ability to amplify the endogenous CAD gene when placed in the drug PALA. Expression of E7, which complexes with a variety of cellular proteins, including Rb, resulted in a p53-independent alteration in cell cycle control, massive cell death and polyploidy upon PALA treatment. These results demonstrate that the viral proteins disrupt cellular processes that safeguard the genome and growth of normal cells.

698 HUMAN SOMATIC MUTATION: INFLUENCES OF GENOTYPE AND CELL LINEAGE ON MUTATION ACCUMULATION *IN VIVO*

Raymond J. Monnat, Jr. *Department of Pathology, University of Washington, Seattle, WA*

Human somatic mutation accumulation may play an important role in the pathogenesis of human neoplastic and degenerative diseases. This presentation will review what we know about quantitative and molecular aspects of human somatic mutation; will discuss recent advances in our understanding of the pathways that control the generation of DNA structural rearrangements in human cells; and will delineate several of the most important conceptual issues and unanswered mechanistic questions concerning the generation and the persistence of mutations in the human body. For this discussion I will draw on work we have done to understand Werner syndrome (WS), an uncommon human autosomal recessive disease that is a caricature of premature aging, is associated with an elevated risk of developing cancer, and displays several different types of genetic instability including a spontaneous deletion mutator phenotype. I will also discuss recent results with a new system we have developed to study quantitative and molecular aspects of somatic mutation *in vivo* in a human epithelial cell lineage.

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699 SHORT-TERM TESTS FOR THE DETECTION OF CHEMICAL INDUCED MITOTIC RECOMBINATION

F K Zimmermann. *Institute for Microbiology and Genetics, Darmstadt Institute of Technology, Darmstadt, Germany.* Sponsor: R H Schiestl

Mitotic or somatic recombination can lead to a loss of heterozygosity, LOH, which causes the expression of recessive tumor alleles and thus can lead to cancer. A considerable part of the mammalian genome is subject to genomic imprinting, a situation where some segments of a chrormosome are inactive when derived from the maternal parent while other segments are inactive when inherited from the paternal parent. Consequently, in certain segments, retention of both parental genomes is required for proper function and LOH can cause characteristic defects. Mitotic recombination has been induced with a wide spectrum of chemicals without any indication of mutagen specificity. This is shown by ionizing radiations which are poor inducers of base substitutions, but extremely efficient inducers of mitotic recombination. Mitotic recombination test systems have been well validated in *Saccharomyces cerevisiae, Aspergillus nidulans* and *Drosophila melanogaster*. Well over 50 chemicals from all categories have been tested in these organisms and shown that assays for the induction of mitotic recombination indeed uncover a wide spectrum of genotoxins. A major problem in genotoxicity testing is caused by the fact that many chemicals are indirect mutagens i.e. the genotoxic effects are only exerted by metabolic derivatives. Predicting human genotoxicity and carcinogenicity requires not only appropriate genetic assays, but also metabolic activation systems which mimick the human conditions. Genes coding for human activating enzymes were expressed in *Saccharomyces cervisiae* to the effect that metabolizing activity could be demonstrated at the biochemical level and mitotic recombination induced.

700 ASSAYS FOR DELETIONS DETECT CARCINOGENS

R H Schiestl. *Department of Molecular and Cellular Toxicology, Harvard School of Public Health, Boston, MA*

DNA deletions are involved in carcinogenesis and in most genetic diseases. Assays scoring for deletions have been constructed and/or used in yeast, in human cells and *in vivo* in mice. In all three organisms deletion events are inducible by a variety of carcinogens including carcinogens that are negative in the Salmonella (Ames) assay and in most other short term tests. Of 47 chemicals of known carcinogenicity status the yeast DEL assay detects 85% correctly. In comparison, the Salmonella assay that detects only 34% correctly. Salmonella positive carcinogens induce DEL recombination with a linear dose response starting at several orders of magnitude below the lowest cytotoxic dose. On the other hand, Salmonella negative carcinogens show a threshold and induce DEL recombination only at high, already cytotoxic doses. Salmonella negative carcinogens induced oxidative stress whereas the Salmonella negative carcinogens did not. These results suggest that many Salmonella negative carcinogens may be active only at high, cytotoxic doses and may be indirect acting. DNA double strand breaks initiated DEL recombination. Alkylating agents like 4-NQO required DNA replication to induce deletions whereas agents directly causing double-strand breaks like X-rays induced deletions in the absence of DNA replication. The DEL assay in human cells is much more sensitive to some chemicals than the yeast DEL assay. Mouse DEL assays may detect tissue specific effects and allow *in vitro - in vivo* comparisons. More widespread use of the DEL assays may reduce the number of experimental animals required, may lead to a higher accuracy in predicting the carcinogenic potential of chemicals and may give more mechanistic information about the biological activity of carcinogens than current assays do.

701 MECHANISM OF PARTICLE-INDUCED INFLAMMATORY CYTOKINE GENE EXPRESSION AND PRODUCTION IN RAT ALVEOLAR MACROPHAGES

M I Luster and W Dong. *Environmental Immunology and Neurobiology Section, NIEHS, Research Triangle Park, NC*

Inflammatory cytokine gene expression and production induced by urban air (A1648) and diesel (D1651) particles were studied in rat alveolar macrophages. Tumor necrosis factor alpha (TNFα) and interleukin-6 gene expression and TNFα secretion were increased in cells treated with 50 to 200 μg/ml Particle A1648 in a dose-response manner. There was no increase in cytokine messenger or production by Particle D1651. A1648-induced increased in TNFα production was not significantly reduced by antioxidants such as TMTU or DMSO. However, the above effect was completely reduced by polymyxin B, an agent that is capable of neutralizing bacterial LPS activities. Endotoxin was found in A1648-treated culture supernatant using *Limulus* amebocyte lysate test, but not in D1651 treated one. We conclude that the mechanism of A1648-induced elevation of cytokine gene expression and production in rat alveolar macrophages was due to the presence of endotoxin incorporated on the particle.

702 DOSE-DEPENDENT PULMONARY FUNCTION CHANGES AND INFLAMMATION FROM INHALATION OF NEAT AND IN-USE MACHINING FLUIDS

J A DeKoster and P S Thorne. *Dept. of Preventive Medicine & Environmental Health, the University of Iowa, Iowa City, IA*

Acute inflammation and pulmonary function responses following inhalation exposure to neat and in-use metal working fluids (MWF) were investigated in guinea pigs and mice. Aims of the study were to characterize physiologic and biomarker responses to inhaled MWF, identify potential causative agents, and evaluate the potential for development of pulmonary hypersensitivity from constituents of in-use MWF. Inhalation exposures of guinea pigs to MWF demonstrated dose-dependent increases in respiratory rate and decreases in breathing volume that served to quantify MWF potency. In-use MWF were more toxic than their corresponding neat MWF. Lowering the pH of neat MWF or spiking neat MWF with active cultures of *Pseudomonas pseudoalcaligenes* did not increase potency to the level of the in-use MWF. These studies showed that significant predictors of respiratory responses were in-use over neat MWF ($p=0.0001$), MWF dose ($p=0.022$), MWF type ($p=0.031$), and the particular MWF sample tested ($p=0.032$). Inhalation exposures to biocides used in the MWF resulted in dose-dependent decreases in respiratory rate. The in-use MWF had endotoxin concentrations from 280

to 1.7 x 10^5 EU/ml. To investigate the role of this endotoxin, inhalation experiments were performed in normal mice (SEN) and endotoxin resistant mice (C3H/HeJ) (RES). These revealed a dose dependent 10,000-fold increase in neutrophils in BAL fluid and a 100-fold increased concentration of IL-6 and TNFα in SEN but not RES mice with exposure to in-use MWF. Smaller increases were seen for IL-1α. Inflammatory responses were not observed with exposure to neat MWF or with sham exposure. Removal of microorganisms from the MWF by filtration of the in-use MWF did not change the responses observed in either strain of mouse. These studies demonstrated that lung inflammation was an important outcome from exposure to in-use MWF and that the endotoxin in these fluids was an important toxicant.

703 EXPOSURE OF HUMAN BRONCHIAL EPITHELIAL CELLS (BEAS-2B) TO OCCUPATIONAL ALLERGENS INDUCES IL-6 PROTEIN SECRETION

P Stetkiewicz and M Wills-Karp. *Department of Environmental Health Sciences, Johns Hopkins University, Baltimore, MD.* Sponsor: T Sutter

Occupational asthma is defined as reversible airways obstruction induced by fumes, vapors or gases encountered in the workplace. The mechanism by which the occupational allergens, tolueue 2,4-diisocyanate (TDI) and nickel sulfate ($NiSO_4$) cause occupational asthma are unknown, although it is thought that there is an immunologic and nonimmunologic component to the disease. Interleukin (IL)-6 is a well known proinflammatory cytokine that has been implicated in the asthmatic inflammatory response. Airway epithelial cells have the ability to secrete several cytokines, including IL-6. We hypothesize that the airway epithelial cells are playing an immunoregulatory role to inhaled allergens by secreting cytokines such as IL-6 that influence the immune system, creating an allergic response to the agent. To determine whether airway epithelial cells release IL-6 upon exposure to occupational allergens, we measured IL-6 levels in the supernatants of cultured BEAS-2B cells by ELISA. Cells were cultured in a defined Ham's F-12 medium plus growth factors, then exposed to either basal Ham's F-12 medium as control, 0.3 um TDI or 10 ug/ml $NiSO_4$ for 30, 60, 90 and 180 minutes. The control cells constitutively released IL-6 protein. The occupational allergen TDI caused a 2 to 3 fold increase over control at 30 and 60 minutes while $NiSO_4$ caused a 2 to 3 fold increase at 30, 60 and 90 minutes. These results suggest that IL-6 is quickly released from BEAS-2B cells in response to these chemical stimuli, suggesting that airway epithelial cells may contribute to the genesis of local inflammatory responses and the pathogenesis of occupationally induced asthma. Supported by HL0734-14 and Johns Hopkins Center for Alternative to Animal Testing (CAAT).

704 DIESEL EXHAUST PARTICLES STIMULATE THE SECRETION OF INTERLEUKIN-1, BUT NOT TUMOR NECROSIS FACTOR-ALPHA, IN RAT ALVEOLAR MACROPHAGES

H-M Yang[1], J Y C Ma[2], V Castranova[2], and J K H Ma[1]. [1]*School of Pharmacy, West Virginia University,* [2]*DRDS, NIOSH, Morgantown, WV*

Exposure to diesel exhaust particles (DEP) in animals results in persistent lung inflammation. This study investigated the effects of DEP on release of proinflammatory cytokines, interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α), by rat alveolar macrophages (AM) *in vitro*. AM were isolated from male Sprague-Dawley rats (~300 g) and incubated with 0, 5, 10, 20, 50 or 100 μg DEP/10^6 AM/ml at 37°C in 5% CO_2. Methanol was used to extract organic compounds from DEP. In concurrent experiments, the effects of washed DEP and methanol DEP extracts of equivalent concentrations were also tested. Twenty-four hours after exposure, AM supernatants were collected and IL-1 and TNF-α activities were determined, respectively, by a mouse thymocyte proliferation assay and a L929 cytotoxicity assay. Exposure to either DEP, washed DEP or methanol DEP extracts did not significantly affect AM viability, as indicated by the results of trypan blue exclusion and LDH release. DEP (at 50 and 100 μg) and methanol DEP extracts (at 20 and 100 μg) significantly increased IL-1 release from AM, while washed DEP was ineffective. In contrast, neither DEP, washed DEP nor methanol DEP extracts stimulated TNF-α production. These results show that DEP can directly activate rat AM to release IL-1. This effect may be attributed to the organic components associated with DEP. Since DEP did not stimulate TNF-α production, IL-1 may play the more important role than TNF-α in DEP-induced inflammation.

705 TEMPORAL EXPRESSION AND CELLULAR DISTRIBUTION OF PULMONARY FIBRONECTIN GENE INDUCTION FOLLOWING EXPOSURE TO AN EMISSION SOURCE PARTICLE

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Fibronectin (Fn) is an extracellular matrix protein involved in a variety of cellular functions including inflammation, cell proliferation, and fibrosis. Previous studies from this laboratory have demonstrated an increase in Fn mRNA in rat lungs after exposure to an emission source particle, known as residual oil fly ash (ROFA). To understand the temporal and spatial distribution of Fn gene expression in ROFA-induced lung injury, we examined Fn mRNA expression by *in situ* hybridization in rat (Sprague-Dawley) lung during the acute phase of lung injury occurring 6 hr to 72 hr following intratracheal instillation of ROFA, 8.3 mg/kg body weight. The pattern of Fn mRNA expression was compared with histopathological changes. Fn mRNA was undetectable in rat lungs treated with saline as well as in lung tissue examined at 6 hr after ROFA treatment. However, at 24 hr after ROFA instillation, Fn mRNA was induced in airway epithelial cells, especially at terminal bronchioles. Histopathology showed peribronchial and perivascular inflammation as well as focal edema. At 48 hr after ROFA exposure, diffusive inflammation in the alveolar region with limited expression of Fn mRNA was evident occurring mainly in proliferating alveolar type 2 epithelial cells. At 72 hr after exposure, extensive Fn mRNA expression was seen in proliferating fibroblasts as well as in hyperplastic and hypertrophic epithelial cells within fibrotic lesions, while the intensity of expression in airway epithelial cells was decreased. Therefore, Fn mRNA induction in the lung following ROFA exposure was associated with inflammatory and fibrotic lesions indicating its possible role in the initiation of fibrogenesis. (This abstract does not necessarily reflect EPA policy).

706 EARLY ALTERATIONS IN THE mRNA ABUNDANCE OF IL-1β, IL-1β, iNOS, MIP-2, TGFβ$_1$, AND VEGF ASSOCIATED WITH ULTRAFINE PARTICLE EXPOSURE

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Polytetrafluoroethylene PTFE (Teflon®]) fume consisting of ultrafine particles (~20 nm) has been described to induce acute effects in the respiratory tract such as hemorrhagic inflammation with extensive pulmonary edema. The ultrafine particles were generated by heating PTFE to ~460°C. Fischer-344 rats were exposed for 15 min. to a concentration of 5 x 10^5 particles/cm^3. Rats were examined 4 hrs. post-treatment. Cells in lung lavage from these rats consisted of 55–75% PMNs. Increases in message abundance for interleukin-1 α and β (IL-1α, IL-1β), inducible nitric oxide synthase (iNOS), and macrophage inflammatory protein-2 (MIP-2) were ~4, 8, 4 and 40-fold, respectively. Transforming growth factor beta-1 (TGFβ$_1$) was unaltered and vascular endothelial growth factor (VEGF) mRNA was decreased by 80% as compared to sham-exposed controls. *In situ* hybridizations demonstrated that PMNs and macrophages as well as epithelial and endothelial cells were actively involved in message production. These results suggest inhaled ultrafine particles penetrate into and interact with alveolar epithelial cells which are activated to induce pro-inflammatory cytokines (IL-1α, IL-1β, MIP-2) and release NO· *via* induction of NOS. VEGF is down regulated and may affect the integrity of the capillary endothelium and result in increased permeability which may also facilitate MIP-2 initiated transmigration of PMNs into the alveolar interstitium. Support by grant NAGW-2356.

707 SILICA ACTIVATION OF MIP-2 AND CINC GENE EXPRESSION BY RAT LUNG EPITHELIAL CELLS: ROLE OF OXIDATIVE STRESS

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Inflammatory mineral particles and fibers activate rat lung epithelial cells *in vitro* to express the neutrophil chemotactic cytokine, macrophage inflammatory protein 2 (MIP-2). Analysis of the 5' flanking region of the MIP-2 gene has demonstrated that the oxidant sensitive transcription factor, NFκB, plays a key role in upregulating MIP-2 expression in response to several agonists including endotoxin and TNFα. In the present studies, we investigated the potential role of oxidant stress and activation of NFκB in silica-induced increases in MIP-2 expression by lung epithelial cells. Briefly, cultures of the rat lung epithelial cell line, RLE-6TN, were exposed to doses of 2–20 μg/

cmA2 silica or the relatively innocuous dust, titanium dioxide, in the presence or absence of hydroxyl radical scavengers. After 6h of exposure, expression of MIP-2 or the gene for a related cytokine, CINC, was assessed using RT-PCR techniques. In addition, nuclear proteins were isolated from the exposed cells and gel mobility shift assays run to characterize NFκB binding activity. *In vitro* silica exposure resulted in dose-related increases in MIP-2 and CINC gene expression; treatment with titanium dioxide had minimal or no effect. Concurrent with increases in MIP-2 expression were increases in nuclear NFκkB binding activity. Addition of the hydroxyl radical scavengers DMSO, TMTU, ethanol or mannitol attenuated the silica-induced increases in MIP-2, CINC and nuclear NFκkB activity in a dose-related manner. These results support a role for oxygen radicals in silica-induced activation of MIP-2 and CINC gene expression. In addition, they suggest that induction of MIP-2/CINC may depend on an oxidant sensitive, NFkB mediated pathway.

708 QUINONE-INDUCED ELEVATION OF MACROPHAGE INFLAMMATORY PROTEIN-2 mRNA IN ALVEOLAR MACROPHAGES

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Neutrophil influx and activation are involved in the pathogenesis of inflammatory lung diseases, including adult respiratory distress syndrome, asthma and chronic bronchitis. Recruitment of neutrophils into the lung is a complex process that depends, in part, upon the local expression of appropriate pro-inflammatory chemokines. Our previous work demonstrated that macrophage inflammatory protein-2 (MIP-2), a member of the CXC subfamily of chemokines, contributes to neutrophil chemotaxis and activation and is induced by bacterial endotoxin in macrophages. The objective of this study was to test the hypothesis that an oxidative stress triggers expression of MIP-2 mRNA and thus, contributes to early inflammation. A rat alveolar macrophage cell line (NR8383) was exposed to menadione (2-methyl-1, 4-naphthoquinone), a quinone compound which undergoes redox cycling and generates reactive oxygen species continuously. Steady state mRNA levels encoding MIP-2 were markedly increased in these cells after 1 h exposure to menadione (25 or 50 μM), remained higher than control levels after 4 h and decreased after 6 h. Menadione-induced up-regulation of MIP-2 mRNA was suppressed by co-treatment with N-acetylcysteine, a synthetic antioxidant. Co-treatment with actinomycin D abolished the induction of MIP-2 mRNA, suggesting that the elevation of mRNA in response to quinone induced oxidative stress was through transcriptional activation of the MIP-2 gene. (Supported by ES05703, ES05947, ES00002 and HL19170)

709 NUCLEAR TRANSCRIPTION FACTORS IN ASBESTOS-MEDIATED LUNG INFLAMMATION

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Studies on the pathophysiology of asbestos - related diseases have indicated the importance of proinflammatory cytokines, chemotactic peptides and certain growth factors, released from asbestos target pulmonary cells. Cytokine gene expression is regulated at several levels, but general control is accomplished by sequence-specific DNA binding proteins, referred to as transcription factors, to the gene promoter or enhancer regions. We studied asbestos-induced modulation of transcription factors activity in lung epithelial cells using in vitro model system. Pulmonary type II-like epithelial cells (A549) following asbestos exposure produced IL-8 and IL6. Coordinated expression of these cytokines, often observed in various inflammatory conditions has been related to common molecular regulation. The binding sites for NF-kB, NF-IL-6 in the both promoters need to be occupied for optimal expression. Asbestos strongly stimulated binding activity of NF-kB and NF-IL-6 to their binding sites in IL-8 promoter. The binding activity to IL-8 promoter required cooperative interactions between NF-kB and NF-IL-6. Asbestos induced binding activity to NF-IL-6 consensus binding site and to NF-kB-like binding sites in IL-6 promoter. Both transcription factors NF-kB and NF-IL-6 respond to oxidative stress, since hydrogen peroxide and hypoxanthine-xanthine oxidase, generating hydrogen peroxide and superoxide anion, upregulated binding activity to NF-kB and NF-IL-6 binding sites in IL-8 and IL-6 promoters. The results demonstrated that asbestos fibers are strong activator of transcription factors and asbestos-induced oxidative stress is involved in transcription factors stimulation. This may be the basis for asbestos affecting the inflammatory cytokine network in the lung.

710 MOLECULAR MECHANISMS OF IMMUNOLOGICAL ACTIVATION OF TYPE II PNEUMOCYTES BY OZONE

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In response to ozone inhalation, pulmonary type II cells become immunologically activated. Our laboratory has been interested in examining the role of activated type II cells in inflammation. In particular, we are investigating molecular mechanisms regulating nitric oxide production by type II cells during lung inflammation. We found that type II cells isolated from control rats readily respond to inflammatory cytokines such as interferon-γ by expressing nitric oxide synthase, a response that is markedly enhanced in cells isolated from rats treated by inhalation of ozone (2 ppm, 3 hr). Type II cells isolated from ozone-treated, but not control rats, were also stimulated to produce nitric oxide following treatment with interferon-γ *in vitro*. The promoter region for the nitric oxide synthase gene is known to contain consensus sequences for several *cis*-acting regulatory factors including NfkB and STAT-1. We found that interferon-γ treatment of type II cells from both control and ozone-treated animals stimulated nitric oxide production by the cells. Treatment with pyrollidine dithiocarbamate, an inhibitor of NfkB activity prevented this response indicating that activation of this factor is important in nitric oxide production by type II cells. Interferon-γ treatment of type II cells isolated from control animals was found to activate STAT-1. Unexpectedly, this transcription factor was found to be constitutively activated in type II cells isolated from ozone-treated rats. Based on these observations, we speculate that both NfkB and STAT-1 transcription factors are important for induction of nitric oxide synthase in type II pneumocytes, and that irritant-induced activation of STAT proteins may, in part, result in enhanced nitric oxide production by type II cells following ozone exposure. (Supported by ALA-NJ and ESO4738.)

711 EFFECTS OF TETRANDRINE ON MACROPHAGE PRODUCTION OF CYTOKINES RESULTING FROM SILICA OR BLEOMYCIN EXPOSURE

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Alveolar macrophage (AM) derived cytokines including interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) play an important role in the development of pulmonary fibrosis. Tetrandrine (TT) has been shown previously to reduce silica-induced pulmonary cell damage and collagen formation. The present objective was to investigate the effects of TT on AM production of IL-1 and TNF-α in response to silica or bleomycin (BLM). AM exposed to silica in vitro exhibited increased release of IL-1 and TNF-α. This increase was effectively reduced in the presence of TT. For in vivo studies, rats were intratracheally instilled with saline as control, 20 mg silica, or 1 mg/kg BLM. TT was administered orally (18 mg/kg) twice before exposure and 3 times/week after exposure for desired time periods. These studies showed that in vivo exposure to silica or BLM yielded AM which produced elevated levels of IL-1 and TNF-α in culture. This production was inhibited by oral treatment with TT. These results indicate that TT treatment inhibits the silica- or BLM-stimulated production of IL-1 and TNF-α. These effects may explain in part the ability of TT to decrease silica induced fibrosis.

712 EARLY INFLAMMATORY RESPONSES TO ADENOVIRAL VECTORS FOR PULMONARY GENE THERAPY

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Cystic Fibrosis (CF) is the most common lethal inherited disease of Caucasians and leads to death from chronic respiratory disease in early adult life in 95% of patients. CF patients suffer from persistent bacterial pulmonary infections and inflammation. The discovery of the CF gene and recognition that the cellular defect can be corrected has opened the door for CF gene therapy. Recombinant adenoviral vectors (Ad) have shown promise for pulmonary gene transfer for CF. However, cellular and humoral immune 2responses to Ad have been problematic and there is evidence inactivated Ad may be proinflammatory. Further, the lipopolysaccharide (LPS) present in the lungs of CF patients may contribute to this inflammation. C3H/HeJ mice (RES) that are hyporesponsive to LPS and normo-responsive C3H/HeBFEJ mice (SEN) were studied to characterize early inflammatory events following Ad intratracheal instillation with or without pretreatment with inhaled-LPS (0.1–8 μg/m^3, MMAD = 1.2 μm, σ_g = 1.8). Dose-response and

time course studies demonstrated that 10^{10} inactivated Ad particles induced striking increases in BAL fluid concentration of TNFα and IL-6 at 3–6 hr and profound BAL neutrophilia maximal at 12–24 hr. SEN mice demonstrated a 10-fold greater response than RES mice at 6, 12, and 24 hr. By 48 hr the neutrophilia was still elevated compared to unexposed control mice. Mice exposed to Ad alone, LPS alone, or Ad + LPS demonstrated significant inflammation at the 3 hr time point as demonstrated by BAL fluid neutrophils, TNFα, and IL-6. In all 3 groups the SEN mice demonstrated a 5- to 300-fold greater response than the RES mice. At 24 hr post exposure significant differences between the SEN and RES mice remained for the treatment groups that received LPS alone or Ad + LPS. These data demonstrate that inactivated Ad does induce early inflammation in the absence or presence of LPS.

713 METALLOTHIONEIN (MT) INDUCTION IN CHANNEL CATFISH FOLLOWING TREATMENT WITH COPPER SULFATE

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Copper sulfate is regularly used in the aquaculture industry for the treatment of various ectoparasitic infestations. Male and female Channel catfish (*Ictalurus puctatus*) were treated for 10 weeks with three levels of copper sulfate (1.7, 2.7, and 3.6 mg/L). No mortality was seen at 1.7 mg/L copper sulfate. However, at the 2.7 mg/L dose, 20 females and 37 males died, and at 3.6 mg/L, 58 females and 73 males died. Livers were excised from survivors at two week intervals and immediately frozen. A cytosolic fraction was prepared from each sample and run on a Sephadex G-75 column to isolate fractions containing metallothionein (MT) which was subsequently measured using a Cd-saturation assay. Body and liver weights, body lengths, sex, and liver somatic indices were also determined for each fish and compared with MT expression. MT expression showed an increasing trend in each does group through the 6-week time point, followed by a general decrease toward control levels after this time. At 6 weeks, fish treated with 1.7mg/L and 3.6 mg/L showed a level of MT expression significantly different from that of controls. At 8 weeks, only the 2.7 mg/L dose group was significantly different, and at 10 weeks, the 2.7 and 3.6 mg/L groups showed significant differences. Correlation coefficients were determined comparing the whole animal measurements with MT expression. A significant inverse correlation with MT was seen only with male body weight ($p = 0.009$, $r^2 = 0.56$) and with male length ($p = 0.017$, $r2 = 0.50$). The results of this study indicate potential differences in hepatic MT expression and whole animal responses to copper in male and female catfish.

714 BASAL AND TRANSIENT, METAL-INDUCED METALLOTHIONEIN LEVELS IN HUMAN AND RODENT CELL LINES AND RESISTANCE TO CADMIUM, CISPLATIN, CHLORAMBUCIL, AND IONIZING RADIATION

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Metallothioneins (MTs) are induced by metal salts, and are implicated in resistance to both toxic drugs and metals. To explore the role of metal induction in cross-resistance to toxic chemotherapeutic agents, we transiently induced MT expression with cadmium or zinc salts and measured resistance to cadmium, cisplatin, chlorambucil, and ionizing radiation in CHO cells and a human monocytic leukemia cell line (THP-1). Metal induction increased MT mRNA and protein accumulation, and enhanced resistance to all toxic treatments, by 4 hours. MT protein and mRNA levels decreased to close to uninduced levels, and enhanced resistance disappeared, by 48 hours. Therefore, metal-induced MT expression is associated with cellular drug and radiation resistance, and transient induction of drug resistance by metal salts may be important in chemotherapy. In addition, a panel of human tumour cell lines with basal, uninduced MT protein levels that varied over a 15-fold range exhibited varying resistance to the toxic chemotherapeutic agent cisplatin. Resistance correlated well with MT level. However, for both metal-induced, and variant, uninduced basal MT, there was only a 2-fold range in drug resistance in spite of the 15-fold range in MT expression. Therefore, while increased MT expression was indicative of increased resistance to chemotherapeutic agents and ionizing radiation, measured differences in MT level may not accurately reflect the degree of cisplatin resistance. In conjunction with data from MT gene transfection studies, these data suggest that MT is a marker of enhanced cellular drug and radiation resistance, but may not independently mediate that resistance.

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715 A SCREENING METHOD FOR NEW METALLOTHIONEIN INDUCERS

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Metallothionein (MT) can be an adjunct in cancer chemotherapy by reducing side effects of anticancer agents. Since human tissues have relatively high background level of MT, and do not efficiently respond to metallic inducers of MT, it is desirable to find a novel non-metallic MT inducer for clinical use. Attempted to develop a screening method to examine **MT**-inducing ability of chemicals, mouse **MT-I** promoter was fused to lacZ gene in a plasmid, $_p$CH110. This plasmid was introduced into Ltk cells, and a clone, L13–17, which responded to **MT** inducers and expressed β-galactosidase (β-Gal) activity, was established. Approximately 200 samples including natural products and synthetic chemicals were examined *in vitro* using L13–17 and a cyclic peptide (PF1070) a product of *Humicola* sp. was selected as a substance which markedly activates **MT** promoter. PF1070 induced β-Gal as efficiently as zinc and the extent of induction was more than additive when it was added into the medium together with zinc or dexamethasone, suggesting that PF1070 activates **MT** promoter by a different mechanism from that of zinc or dexamethasone. To identify elements in the promoter activated by PF1070, various **MT-I** promoters having different length of deletions from the 5' end were prepared and fused to lacZ. The results obtained by using these deleted promoters indicate that the induction by PF1070 is not mediated by **GRE** and that the region around -150 position in MT-I promoter plays an important role in the induction of **MT** by PF1070.

716 ENHANCED RENAL TOXICITY BY INORGANIC MERCURY IN METALLOTHIONEIN-NULL MICE

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To elucidate a physiological role of metallothionein (MT) in the manifestation of inorganic mercury toxicity, we have studied the susceptibility of MT-null transgenic mice, kindly provided by Dr. A. Choo (Murdoch Institute for Research into Birth Defects, Royal Children's Hospital, Australia), to the renal toxicity of mercuric chloride ($HgCl_2$). Nine-week-old female C57BL/6J mice, which were used as a wild type control, and female MT-null mice of the same age (4 mice for each group) were given s.c. injections of $HgCl_2$ at doses between 20 and 50 μmol/kg b.w. Blood was collected from each mouse under diethylether anesthesia, 3 days after the injection of $HgCl_2$. Plasma BUN and creatinine values were determined as indicators of nephrotoxicity. The basal MT level in the kidney of MT-null mice was negligible by MT RIA, and approximately 5.0 μg/g tissue in C57BL/6J mice. In the MT-null mice, BUN and creatinine values were significantly elevated at doses >30 μmol/kg, and they were significantly higher than those of C57BL/6J mice, which only markedly increased at a dose of 50 μmol $HgCl_2$/kg. 50% of MT-null mice injected with 50 μmol/kg of $HgCl_2$ had died within 3 days, although all of C57BL/6J mice in this study survived. In the present study, we found that $HgCl_2$ caused severe kidney damage in MT-null mice compared to C57BL/6J mice. We show that MT is a major determinant for the threshold mercury level for the renal toxicity.

717 GLUTATHIONE DEPLETION ENHANCES CADMIUM-INDUCED CYTOTOXICITY BUT BLOCKS PROTO-ONCOGENE ACTIVATION IN RAT L6 CELLS

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Cadmium (Cd) is a highly toxic metal and a known carcinogen. Although the carcinogenic mechanism of Cd is unknown, the metal induces transcriptional activation of certain proto-oncogenes, including c-*myc* and c-*jun*, in rat myoblast (L6) cells. The extent of Cd-induced oncogene expression is limited by the presence of metallothionein (MT), a inducible Cd-binding protein.

Glutathione appears to play a critical role in protection against Cd intoxication before the onset of MT synthesis. Thus, we studied the effects of glutathione depletion on Cd-induced cytotoxicity, oncogene activation, and MT synthesis in L6 cells after pretreatment with L-buthionine sulfoximine (BSO), a potent inhibitor of γ-glutamyl cysteine synthetase. Exposure to BSO (5 and 25 μM) resulted in dose-dependent decreases in cellular glutathione. Although BSO was not itself cytotoxic in L6 cells, BSO pretreatment resulted in a dose-dependent increase in the sensitivity to Cd (0.35 to 7.14 μM $CdCl_2$) cytotoxicity, as assessed by the tetrazolium-based dye assay. Glutathione depletion had no effect on Cd-induced MT synthesis. Cd, at non-toxic levels (1 μM), effectively activated both c-*myc* and c-*jun* expression as assessed by increases in gene specific mRNA levels. BSO treatment (5 μM) similarly caused an increase in expression of c-*myc* and c-*jun*. However, BSO pretreatment markedly decreased levels of c-*myc* and c-*jun* mRNA transcript after Cd (1 μM) treatment. These results suggest that increased cytotoxicity resulting from the combined exposure abrogates the oncogene activation observed after either treatment alone, and indicate that proto-oncogene activation is independent of Cd-induced cytotoxicity.

718 Cd^{++}- BINDING METALLOTHIONEIN INDUCED BY ARSENIC & SELENIUM IN THE EARTHWORM, *Lumbricus*

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Metal ions such as Cu^{++}, Zn^{++} and Cd^{++} have been shown to induce biosynthesis of metallothionein (MT) in several species of invertebrates including the common earthworm, *L. terrestris*. Similar to mammalian MTs, two isoforms of MT (MT-1 and MT-2) were also identified in the earthworms. Inorganic and organic arsenicals are found to induce MTs in mice. Selenium compounds, however, failed to initiate biosynthesis of MTs in rats and monkeys. By injecting low doses of sodium selenite and sodium arsenite into the coelomic cavities of the earthworm, we induced the biosynthesis of a heat stable, low molecular weight protein, which shows a light absorption peak at 254 nm. This protein also exhibits strong affinity to radioactive $^{109}Cd^{++}$. MT can be induced by Se is shown for the first time. It's affinity to Cd may have implications in detoxification of excess Cd with low doses of Se or As.

719 METALLOTHIONEIN(MT)-NULL MICE ARE SENSITIVE TO CISPLATIN-INDUCED NEPHROTOXICITY AND HEPATOTOXICITY

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MT has been proposed to play an important role in protection against toxic side effects of the anticancer drug cisplatin. To determine whether constitutive levels of MT protect against cisplatin, the cisplatin toxicity in control and MT-I and II knock-out (MT-null) mice were compared. Control (129/Ola x C57BL/6J) and MT-null mice were injected with cisplatin (20–50 μmol/kg, *ip*). Kidney injury was evaluated 24–96 hr after cisplatin administration by quantification of blood urea nitrogen (BUN), as well as by histopathology. MT-null mice were more sensitive than controls to cisplatin-induced renal injury. Seventy-two hr after cisplatin (50 μmol/kg) administration, BUN concentrations were increased 3.5-fold in control mice, as compared to 6.5-fold in the MT-null mice. MT-null mice were also more sensitive than controls to cisplatin (200 μmol/kg)-induced liver injury, as evidenced by serum alanine aminotransferase activity and histopathology. This dose of cisplatin was lethal to 17% of control mice, but 57% of the MT-null mice died. Furthermore, MT-null hepatocytes were more sensitive to the cytotoxicity of cisplatin (50–200 μM) than those of controls. These results suggest that constitutive MT levels decrease the sensitivity of mice to cisplatin toxicity. (Supported by NIH Grant ES-01142)

720 HEPATOCYTES OF METALLOTHIONEIN-NULL MICE ARE SENSITIVE TO THE TOXICITY OF CADMIUM AND *tert*-BUTYLHYDROPEROXIDE

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Metallothionein (MT) has been proposed to play an important role in heavy metal detoxication and in the scavenging of free radicals. Effects of MT on the cytotoxicity of cadmium (Cd), *tert*-butylhydroperoxide (tBHP) and *N*-methyl-*N*-nitroso-guanidine (MNNG) were examined using primary hepatocyte cultures from control (C57BL/6J) and MT-I and II knock out (MT-null) mice. Compared to controls, MT-null hepatocytes had little Cd-binding proteins (MT equivalents), but the level of cellular glutathione was similar. MT-null hepatocytes were more sensitive than controls to the cytotoxic effects of Cd (2.5–300μM) and tBHP (100–500 μM), as indicated by the release of lactate dehydrogenase into the medium. Cd and tBHP also produced more lipid peroxidation in MT-null hepatocytes than in control cells, as indicated by the abundance of thiobarbituric acid-reactive substances. However, MT-null hepatocytes were equally sensitive as controls to the toxicity of MNNG (0.5–2 mM), suggesting that MT does not protect against MNNG-induced DNA damage. These results support the hypothesis that constitutive MT levels affect the sensitivity of mammalian cells to Cd and oxidative stress, but not to all genotoxic chemicals. (Supported by NIH Grant ES-01142)

721 CADMIUM-INDUCED TESTICULAR INJURY IN METALLOTHIONEIN-NULL MICE: ROLES OF MOUSE STRAIN AND METALLOTHIONEIN

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Parenteral administration of cadmium (Cd) to male mammals causes testicular damage. Testicular metallothionein (MT), a Cd-binding protein, has been proposed to protect against this injury. In this study, MT-I and II null (MT-null) mice were used to examine the role of MT in Cd-induced testicular damage. MT-null (129xC57 background) and control (129xC57; 129x129 and C57xC57) mice were given $^{109}CdCl_2$ (5–30 μmol/kg, ip), and testicular injury was determined 24 hr later by histopathology and heme concentration. Cd produced dose-dependent testicular injury in all strains of mice except the C57xC57 strain, although testicular Cd-binding proteins and ^{109}Cd content were similar in these control strains. The MT-null mice were more sensitive than controls to Cd-induced testicular injury. At the dose of 10 μmol Cd/kg, the heme content in the MT-null mice was about twice that in 129xC57 and 129x129 mouse strain, 5–7 times more than the C57xC57 strain, in spite of similar ^{109}Cd content in their testes. These data indicate that the genetic resistance of C57 mouse strain to Cd-induced testicular injury is not due to MT, nor to decreased Cd uptake; but suggest that MT does play a partial role in protection against Cd-induced testicular damage. (Supported by NIH Grant ES-01142)

722 CHARACTERIZATION OF CADMIUM BINDING PROTEINS IN THE PLANARIAN, *Dugesia dorotocephla*

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Cadmium (Cd) has been demonstrated to be capable of producing retropharengeal tumors in the planarian, *Dugesia dorotocephla*, but a protein similar to mammalian metallothionein capable of binding heavy metals has never been identified in this species. Planaria were cultured in soft reconstituted water. Treatment groups were exposed to 10 ppb Cd for 10 days. A cytosolic protein fraction from planarian homogenate, isolated by centrifugation, was heat treated at 100°C and then fractionated by gel filtration chromatography. The cytosolic samples were saturated with excess Cd and then treated with Chelex to remove unbound metal before fractionation. Elution of protein fractions was then evaluated by measuring absorbance at 254 nm and 280 nm. Amounts of bound Cd in the fractions were determined by atomic absorption spectrophotometry. A Cd containing protein peak eluted with an apparent molecular weight of approximately 5,500 kD. The amount of Cd binding protein increased with exposure to Cd. Asexual *D. dorotocephla* pretreated with Cd demonstrated a 100% increase in the content of the Cd binding protein fraction, whereas pretreated sexual *D. dorotocephla* exhibited a 65% increase. Planaria have thus been shown to contain a heat stable, low molecular weight protein which binds cadmium. The content of this protein increases with Cd exposure. (Supported in part by NIH grant R03 RR08578-01)

723 THE EFFECTS OF GLUTATHIONE DEPLETION AND METALLOTHIONEIN EXPRESSION ON ARSENITE-INDUCED CYTOTOXICITY AND PROTO-ONCOGENE ACTIVATION *IN VITRO*

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Arsenic compounds are considered human carcinogens. *In vitro*, arsenite (As^{+3}) induces neoplastic transformation associated with activation of proto-oncogenes, such as c-*myc*, and expression of such genes may be involved

in acquisition of malignant phenotype. Arsenic toxicity occurs, in part, by interaction with sulfhydryl groups and glutathione (GSH) is important in As^{+3} detoxication. *In vivo*, zinc (Zn) induces tolerance to As^{+3}. Zn also induces the synthesis of metallothionein (MT), a sulfhydryl-rich, metal-binding protein that often functions in defense against metal toxicity. Thus, we studied the relationship between levels of GSH or MT and As^{+3}-induced cytotoxicity or c-*myc* expression in rat L6 myoblasts *in vitro*. Cytotoxicity was measured by the tetrazolium dye assay and c-*myc* expression was measured by Northern blot analysis of mRNA using a specific oligonucleotide probe with normalization to 18S rRNA. When L6 cells were treated with L-buthionine sulfoximine (BSO, 1, 5 or 25 μM, for 24 hrs), a potent inhibitor of γ-glutamyl cysteine synthetase, a dose-dependent depletion of GSH occurred (62% maximum). As^{+3} exposure (1 to 100 μM, 24 hrs) was up to 40 times more cytotoxic in GSH depleted cells (minimum LC_{50} = 1 μM) than in cells with normal levels of GSH (LC_{50} = 41 μM). Conversely, Zn pretreatment (100 or 125 μM) had no effect on As^{+3}-induced cytotoxicity in L6 cells, although Zn effectively induced MT synthesis (~ 10-fold). As^{+3} (1 to 5 μM) alone activated c-*myc* expression 2–6 hrs after exposure by a maximum of 1.7-fold over control while, in GSH depleted cells, As^{+3} enhanced c-*myc* expression during the same period even further (2.9-fold over control). Zn pretreatment had no effect on As^{+3}-induced c-*myc* activation. These results suggest that the levels of GSH, but not of MT, play an important role in protection against As^{+3} cytotoxicity.

724 STRAIN AND SPECIES COMPARISON OF CADMIUM DISPOSITION AND METALLOTHIONEIN GENE EXPRESSION IN RATS AND MICE

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Depending on the route and duration of exposure, cadmium (Cd) can induce tumors in several tissues, including the lung, or cause hepatic, pulmonary or renal damage. Rats are often more sensitive to Cd than mice, although sensitivity to Cd toxicity may vary widely between strains. The metal-binding protein, metallothionein (MT), is often associated with native or acquired Cd tolerance and the *MT* gene is activated by exposure to metal. Thus, in this study, male WF rats, "Cd-sensitive" C3H mice, and "Cd-resistant" DBA mice received 0, 1, 3, 10 or 30 μmol $CdCl_2$/kg, sc, and 24 hrs later Cd, MT protein and *MT* mRNA levels were measured. Hepatic Cd was similar in all animals while renal Cd levels were similar in rats and DBA mice but were ~ 2-fold higher in C3H mice. MT protein showed dose-related increases in liver and kidney of all animals. C3H mice showed the lowest basal and induced levels of hepatic MT while basal renal MT was much higher in rats than in either mouse strain. Basal and induced levels of pulmonary MT were similar in the mice but were much lower and not inducible by Cd in rats. High basal and induced levels of *MT* mRNA were seen in rat liver, but induced renal levels were much higher in mice. Basal levels of *MT* mRNA in the lung were similar in all of the animals tested but induction in rats was much more pronounced. These results indicate Cd disposition and regulation of *MT* expression varies widely with species and strain and does not necessarily correlate with tissue sensitivity to damage. Thus metal distribution, the inducibility of the *MT* gene and translational efficiency of *MT* mRNA all appear to be important factors in determining sensitivity to Cd toxicity and carcinogenesis.

725 DEVELOPMENTAL EFFECTS OF ATRAZINE AND NONYLPHENOL IN TADPOLES

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Amphibian species have experienced worldwide declines over the past few decades and no single cause has been identified. Adverse effects of environmental contaminants in amphibians are poorly understood and chronic exposure studies are rare. We have examined the effects of atrazine, a widely used herbicide, and nonylphenol, an environmental degradation product of certain detergents, in *Xenopus laevis* tadpoles. Larvae were exposed in a flow-through system until after the completion of metamorphosis. Concentrations of 10, 2 and 1 ppm atrazine significantly delayed development, whereas 0.5, 0.25, and 0.125 ppm did not. Development of sexual characteristics was delayed at all atrazine concentrations tested. Final body weight was significantly reduced at 10, 2, 1, and 0.5 ppm. Triiodothyronine levels measured after 3 weeks of atrazine exposure did not show significant differences between treatment groups and controls. Nonylphenol exposure resulted in a significantly increased proportion of females at nominal concentrations of 100 and 25 ppb, but not at 50, 12.5 or 6.25 ppb. Proper timing of metamorphosis is critical for amphibians whose habitat often consists of seasonal ponds. Delayed development, low body weight, and a skewed sex ratio may imply poor recruitment for an impacted amphibian species.

726 INDOLE-3-CARBINOL AND 2,3,7,8-TETRACHLORODI BENZO-p-DIOXIN (TCDD) REDUCES DAILY SPERM PRODUCTION AND ALTERS EPIDIDYMAL FUNCTION

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A weak Ah receptor agonist such as the naturally occurring "dioxin" indole-3-carbinol (I3C), which is found in cruciferous vegetables, may cause similar adverse effects on the male reproductive system as that of exodioxins such as TCDD. The objective of this study was to determine if I3C or TCDD would alter daily sperm production (DSP), number of epididymal spermatozoa, or epididymal transit time of spermatozoa (ETT). On day 15 of gestation, Sprague Dawley females rats were given a single oral dose of I3C (100 mg/kg; n = 10), TCDD (1.0 μg/kg; n = 13) or equal volume of vehicle (n = 14). Following treatment male offspring were terminated at 63 days of age. Testicular spermatids and epididymal spermatozoa were counted in homogenates to determine DSP and number of epididymal spermatozoa. ETT was calculated by dividing the number of epididymal spermatozoa by DSP/testis. Mean values (±SEM) will be presented for Control, I3C, and TCDD, respectively. There was no effect on testicular weights by these treatments. Both I3C and TCDD reduced ($p<0.01$) DSP/testis and DSP/g (19.4±0.9, 16.7±0.8, and 15.9±0.7 X 10^6). TCDD reduced ($p<0.01$) the epididymal weight and number of epididymal spermatozoa (157.2±8.1, 153.7±5.9, and 109.5±5.1 X 10^6). However, I3C altered ($p<0.01$) total ETT of spermatozoa (5.7±0.2, 6.8±0.3, and 5.4±0.3 days). ETT through the cauda epididymidis was prolonged ($p<0.01$) by I3C and decreased ($p<0.01$) by TCDD (2.8±0.1, 3.6±0.2, and 2.0±0.1 days). Reduced DSP is associated with prolonged ETT in men. Alterations of ETT reduces fertility. In conclusion, I3C reduces male reproductive capacity at least as severely as that of TCDD and thus both natural "dioxins" and exodioxins should be considered in the overall health risk assessment. **NIH RO1 AG 11093-10 and NIH T32 ESO 7273**

727 4-TERT-OCTYLPHENOL: *IN VITRO* AND *IN VIVO* ASSESSMENTS OF POTENTIAL ESTROGENICITY IN RATS

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4-tert-octylphenol (OP), a biodegradation product of alkylphenol polyethoxylates, has been reported to induce estrogenic responses in vitro. We compared the ability of OP, 17β-estradiol (E), and the estrogenic pesticide, methoxychlor (MXC) to inhibit ligand binding to the estrogen receptor (ER) using in vitro competitive receptor binding assays. OP inhibited ER binding (IC_{50} 2.9 μM) but was not as effective a competitor as E (IC_{50} 2nM) or the metabolite of MXC, HPTE (IC_{50} 0.17 μM). The IC_{50} for MXC was 204μM. We also evaluated a number of physiological responses in immature Long Evans and Sprague-Dawley female rats after exposure to MXC (50 mg/kg; gavage; 21– 35 days-of-age) and OP (50, 100 or 200 mg/kg; gavage). MXC and OP (200 mg/kg) accelerated vaginal opening (VO) by 6.8 and 4.6 days, respectively. Exposure to MXC (50 mg/kg) for 3 days increased uterine weight and PR synthesis. OP (up to 500 mg/kg) had no effect on these parameters. In 27-day-old ovariectomized rats, treatment with E or MXC for 7 days induced VO, uterine growth, and elevated pituitary prolactin (prl) concentration. OP (500 mg/kg) induced VO in 4 of 6 animals, but again failed to stimulate uterine growth or prl. These data show that (1) HPTE exhibited a greater affinity than OP for the ER in vitro; and (2) although both OP and MXC accelerated VO, OP failed to induce any other estrogenic responses evaluated using this in vivo testing regime.

728 ASSESSMENT OF CHEMICALS ALLEGED TO POSSESS ESTROGEN RECEPTOR-MEDIATED ACTIVITIES USING *IN VITRO* RECOMBINANT RECEPTOR/REPORTER GENE ASSAYS

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In vitro recombinant receptor/reporter gene assays have been used to assess and rank the potency of chemicals alleged to possess estrogenic activities. The Environmental Estrogen (E2) Bioassay consists of HeLa cells stably transfected with the Gal4-human estrogen receptor chimeric construct, Gal4-HEGO and the Gal4-regulated reporter gene, 17m5-G-Luc. The assay exhibits a 10-fold increase in luciferase activity following treatment with 1.0 nM 17β-estradiol (E2) and an EC_{50} value for E2 of approximately 20 pM. Based on these results, it has been estimated that the E2 Bioassay has a detection limit of approximately 5 pg E2/ml. This assay has been used to examine a number of chemicals including natural products, environmental pollutants and industrially-relevant chemicals for estrogen receptor (ER)-mediated activities. Several chemicals including genistein, naringenin, *o,p'*-DDT, *p*-nonylphenol, Bisphenol A and Endosulphan I significantly induce 17m5-G-Luc activity in a dose-dependent fashion with EC_{50} values ranging from 0.6 to 2.0 μM. In contrast, no significant induction in luciferase activity was observed with Kepone, Toxaphene, Endosulphan II or any of the phthalate esters examined (i.e. dimethyl, diethyl, diethylhexyl, dibutyl, dicyclohexyl, diisoheptyl, dioctyl, diisononyl and diisodecyl). These studies demonstrate the utility of using *in vitro* recombinant receptor/reporter gene assays to identify chemicals that possess activities mediated by the ER.

729 MALE REPRODUCTIVE TOXICITY FOLLOWING PERINATAL EXPOSURES TO THE ANTI-ANDROGEN p,p'-DDT

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Certain chlorinated hydrocarbons are known to disrupt normal endocrine milieu. We have performed the following two exposure studies in Dutch Belted rabbits in an effort to elucidate the mechanisms by which DDT mediates its male reproductive toxicity: 1) gestational plus infantile exposure to p,p'-DDT (dams: 25 mg/kg; pups: 10 mg/kg; by gavage 1x/wk); and 2) infantile exposure alone. Neither exposure caused mortality or morbidity. Infantile exposure alone resulted in delays of up to 6 wk in testicular descent. When combined with gestational exposures, uni/bi-lateral cryptorchidism was observed in addition to the delay in testicular descent. Serum DDT residue levels in offspring following infantile exposures, as measured by gas chromatography/electron capture detection, were 208 ppb DDT and 38 ppb DDE, which rapidly cleared from blood by 12 wk of age. Neither exposure to p,p'-DDT altered serum concentrations of luteinizing hormone and testosterone, or daily sperm production (in animals with scrotal testes). These studies suggest reproductive toxicity in male offspring following perinatal exposures to lower levels of DDT than have been previously reported. Analyses of endocrine-responsive cytochrome P450 gene expression in gonadal tissues are in progress. (Support from College of Veterinary Medicine and Biomedical Sciences, Colorado State University).

730 INFLUENCE OF METHOXYCHLOR ON ESTROGENIC RESPONSES AND CYTOCHROME P450 INDUCTION IN CHANNEL CATFISH (*Ictalurus punctatus*)

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Methoxychlor [1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane], an insecticidal DDT-analog, is characterized as a xenoestrogen by virtue of its ability to induce responses characteristic of estradiol (E2). Mammalian studies have demonstrated that multiple bidaily doses of methoxychlor to female rats induces hepatic microsomal P450. The objective of the present study was to assess relative estrogenic effects of methoxychlor in channel catfish, a representative freshwater teleost. The estrogenic effects of methoxychlor were assessed by evaluating vitellogenin (Vg) induction, estrogen/testosterone ratios, and gonadal somatic index, as compared to E2. In addition, induction of cytochrome P450 enzymes involved in steroidogenesis were examined. Juvenile channel catfish (65 to 95g) were exposed to methoxychlor on day 0 and day 7 by intraperitoneal (i.p.) injection of a 0.25% agar suspension. Appearance of Vg in serum was measured by ELISA 14 days post injection. Hepatic CYP1A and CYP2K-like proteins were characterized 14 days post injection as determined by western blot analysis using rainbow trout CYP1A1 and CYP2K1 antibodies provided by Dr. D. R. Buhler. Methoxychlor treatments of 127 and 380 mg/kg resulted in Vg levels of 2.5 and 3.3 mg/ml, respectively. Vitellogenin appearance in serum indicates that methoxychlor has the ability to induce estrogenic responses in channel catfish.

731 USE OF A YEAST-BASED ESTROGEN RECEPTOR ASSAY TO ASSESS CHEMICAL INTERACTIONS WITH THE ESTROGEN RECEPTOR

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There has been increasing public concern that chemicals in the environment are affecting human health by disrupting normal endocrine function, particularly through interaction directly with hormone receptors to alter endocrine function. In our initial studies, we have used a yeast-based estrogen receptor assay to assess chemical interaction with the estrogen receptor. The yeast transformants used for this assay contain one plasmid that encodes for the human estrogen receptor and a second plasmid that contains two tandemly linked estrogen responsive elements upstream of the β-galactosidase reporter gene. Selected chemicals were added in doses ranging from 10^{-12} to 10^{-5} M to cultures of yeast transformants. Following overnight incubation, the cultures were then assayed for β-galactosidase activity. Diethylstilbesterol, and 17-β estradiol were the most potent at inducing β-galactosidase, followed by the phytoestrogen, coumestrol, which was approximately 100-fold less potent than estradiol. Nonylphenol and bisphenol-A also induced β-galactosidase but are approximately 10,000-fold less potent than estradiol. The pesticide and suspected estrogenic compound, endosulfan, did not induce activity up to a dose of 10^{-5} M and thus would be predicted not to interact with the estrogen receptor. These data demonstrate the utility of this assay for assessing chemical interactions with the estrogen receptor and for determining relative potency. Future studies will determine how well this assay correlates with *in vivo* responsiveness.

732 HYDROXY-PCBs AS ANTIESTROGENS: STRUCTURE-ACTIVITY RELATIONSHIPS

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2',3',4',5'-Tetrachloro-4-biphenylol (Cl_4-4-OHBP) competitively bound to the rat uterine cytosolic estrogen receptor (ER) (IC_{50} = 7.0 × 10^{-5} M) and exhibited weak estrogenic activity. The effects of a single ortho- or meta-chloro substituent on the phenolic ring and variable substitution (2',4',5'-; 2',3',5',6'-; 2',5'-; 2',3',4',6'-) on the nonphenolic ring were investigated for their activities as ER agonists and antagonists. In HeLa cells stably-transfected with a Gal4:human ER chimera and a 17mer-regulated luciferase reporter gene, only two compounds (10^{-5} M), namely 2,2',4',6'-tetrachloro- and 2',3,4',6'-tetrachloro-4-biphenylol induced > 50% of the response observed for 17β-estradiol (E2) (10^{-9} M). However, in cells cotreated with E2 plus the hydroxy PCBs, there was a significant decrease in E2-induced luciferase activity, and the most active compound, 2,2',3',4',5'-pentachloro-4-biphenylol (Cl_5-4-OHBP, 10^{-5} M), inhibited 80% of the induced response. Administration of Cl_5-4-OHBP (5 to 100 mg/kg) to 25 day old female Sprague-Dawley rats resulted in minimal estrogenic effects; however, in rats cotreated with E2 (0.5 μg/rat) plus Cl_5-4-OHBP (50 or 100 mg/kg), E2-induced PR binding and peroxidase activity were decreased. Thus hydroxy-PCBs exhibit structure-dependent ER agonist and antagonist activities.

733 ANTIESTROGENICITY ACTIVITY OF HYDROXYLATED PCBs IDENTIFIED IN HUMAN SERUM

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Several hydroxylated polychlorinated biphenyls (PCBs) identified in human

serum have been synthesized and these include: 2,2',3,4',5,5'-hexachloro-4-biphenylol, 2,3,3',4',5-pentachloro-4-biphenylol; 2',3,3',4',5-pentachloro-4-biphenylol, 2,2',3,3',4',5-hexachloro-4-biphenylol, 2,2',3,3',4',5,5'-heptachloro-4-biphenylol, 2,2',3,4',5,5',6-heptachloro-4-biphenylol, 2,2',3',4,4',5,5'-heptachloro-3-biphenylol. The hydroxy-PCBs (10^{-5} M) exhibited minimal binding to the rat uterine cytosolic estrogen receptor (ER). The estrogenic activity of these compounds was determined utilizing two estrogen-responsive *in vitro* bioassays, namely (i) HeLa cells stably-transfected with a Gal4: human ER chimera and a 17mer-regulated luciferase reporter gene, and (ii) HeLa cells transiently transfected with a full length ER expression plasmid and a plasmid containing an E2-responsive vitellogenin A2 promoter and a chloram-phenicol acetyl transferase (CAT) reporter gene. None of the hydroxy-PCBs (10^{-5} to 10^{-8} M) significantly induced luciferase activity in the stably-transfected HeLa cells and only some compounds induced CAT activity (< 50% of 10^{-9} M E2) at the 10^{-5} M concentration. In cells cotreated with E2 plus different concentrations of the hydroxy-PCBs, all of the compounds were antiestrogenic in at least one bioassay.

734 THE EFFECTS OF PERINATAL/JUVENILE PESTICIDE EXPOSURE ON ADULT REPRODUCTIVE FUNCTION. I.METHOXYCHLOR

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This study used the design of Chapin et al (Toxicologist, 1996) to examine the effects of perinatal and juvenile Methoxychlor (M) exposure on adult reproductive function. Dams were treated from gd14 to pnd7, then pups were gavaged with the same doses (5, 50, or 150 mg M/kg/d) until pnd 42. M150 caused a 17% reduction in live pup number, and a ≤16% weight gain reduction up to weaning. Vaginal opening was accelerated by 3-7 days in all treated females, and prepuce separation delayed by 8-30 days in M50 and M150 males. F_1 ovary weights at pnd42 (end of dosing) were dose-dependently reduced in all M females, by 28-54%. When the F_1 pups were adults, 15/dose group were mated to naive partners. M150 males impregnated 3/30 partners, vs. 21/30 in controls; litter size was unaffected. After the second mating, the females were killed before delivery; pre-implantation loss at M150 was double the control rate. At necropsy, M50 and M150 males had reduced testis and epididymis weights and sperm or spermatid counts, although spermatogenic efficiency was unaffected. Sertoli cell counts are ongoing. Treated females were sterile at M150, while only 2/15 mated at M50. Weight gain of the first F_2 litter to pnd 10 was not different across treatment groups. For the second mating at M50, litter size was 30% less than controls and preimplantation loss was 3-fold the control value. Empty uterus weight was lower than control in all treated females. Adult corpora lutea numbers were reduced at M5 and M50. Histopathologic evaluations are ongoing. This study shows that even 5 mg/kg M during early development produces detectable effects in adult reproductive endpoints.

735 FAILURE OF CHLORO-S-TRIAZINE-DERIVED CHEMICALS TO INDUCE ESTROGEN RECEPTOR-MEDIATED RESPONSES *IN VIVO* AND *IN VITRO*

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The alleged estrogenic activities of atrazine and simazine were investigated *in vivo* using the immature Sprague-Dawley rat uterus and *in vitro* using MCF-7 human breast cancer cells and the estrogen-dependent recombinant yeast strain, PL3. Animals dosed with 50, 150 or 300 mg/kg of atrazine or simazine for 3 consecutive days did not exhibit significant increases in uterine wet weight while decreases in cytosolic progesterone receptor (PR) binding levels and peroxidase activity were observed. 17β-Estradiol (E2)-induced increases in uterine wet weight were not significantly affected by cotreatment with either chemical, however some dose-independent decreases in E2-induced cytosolic PR binding and uterine peroxidase activity were observed. *In vitro*, atrazine and simazine did not affect basal or E2-induced MCF-7 cell proliferation or the formation of nuclear PR:DNA complexes as determined by gel electrophoretic mobility shift assays. In addition, these chloro-S-triazines did not display agonist activity or antagonize E2-induced luciferase activity in MCF-7 cells transiently transfected with a Gal4-human estrogen receptor chimera and a Gal4-regulated luciferase reporter gene. Moreover, the estrogen-dependent PL3 yeast strain was not capable of growth on minimal media supplemented with atrazine or simazine in place of E2. Collectively, these results indicate that the reported estrogenic and antiestrogenic effects elicited by these chemicals are not estrogen receptor-mediated.

736 GENISTEIN TOXICITY IN THE FEMALE RAT REPRODUCTIVE SYSTEM

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The phytoestrogen, genistein, when given neonatally and prepubertally has been demonstrated to suppress chemically-induced mammary cancer in the rat. Since it has been administered early in life we were concerned for its potential to result in adverse effects on the reproductive/endocrine system. Spraque-Dawley CD rats were injected with 500 μg genistein/g b.w. neonatally (days 2, 4 and 6 postpartum) or prepubertally (days 16, 18, and 20 postpartum). Fifty day old female rats treated neonatally with genistein had significantly longer estrus cycles, lower circulating progesterone but not estrogen concentrations, and fewer corpora lutea. While fifty day old female rats exposed prepubertally to genistein had slightly longer estrus cycles, sera estrogen and progesterone concentrations and ovarian follicular development were not significantly altered. We conclude that neonatal but not prepubertal genistein exposure is toxic to the reproductive/endocrine system.

737 LONG-TERM EFFECTS OF GREAT LAKES CARP CONTAINING DIET ON REPRODUCTION IN MICE

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The objective of this study was to investigate the effects of Great Lakes contaminants on reproductive performance and sperm fertilizing ability in B_6D_2-F1 male mice. Pregnant C57BL/6J mice, bread with DBA/2J male, were randomly divided into three treatment groups: C (lab chow with fish oil), I (40% Iowa carp with 60% lab chow) and G (40% Great Lakes carp with 60% lab chow). These diets were of similar nutritional and mineral value. The treatment diets were provided immediately after the dams (F-0) gave birth and were fed through lactation. The offspring (F-1) were continuously fed their respective diets from weaning to termination. F-1 males at 44 weeks of age were paired with non-treated females. Fecundity, litter size, sex ratio, viability index and lactation index were recorded. Sperm fertilizing ability *in vitro* was examined with eggs collected from non-treated females after natural breeding. A computer-assisted program was used to perform sperm motion analysis hourly after epididymal sperm were collected. In the Great Lakes treatment group, high mortality rates were observed in nursing pups and males between the age of 29 to 31 weeks. Decreases in body weights were also observed among these males. At 44 weeks of age fecundity, sperm concentration and sperm fertilizing ability were significantly lower than in C and I treatment groups. Female mice in the Great Lakes treatment group had a delay in vaginal opening and a decrease in body weight. [Supported by Michigan Great Lakes Protection Fund]

738 *IN UTERO* 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) EXPOSURE DECREASES THE NUMBER OF EPITHELIAL BUDS IN THE FETAL RAT PROSTATE

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In utero 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure impairs rat prostate growth very early in development, resulting in decreased prostate weight well into adulthood. The highly branched ducts of the mature ventral, dorsal, and lateral lobes of the rat prostate begin as solid epithelial cords (prostatic buds) that bud from the fetal urogenital sinus between gestation days (GD) 18.5 and 20.5. To determine whether TCDD-induced decreases in prostate weight involve alterations in the epithelial budding process, pregnant Holtzman rats were administered TCDD (1.0 μg/kg, po) on GD 15, and male fetuses were caesarean delivered on GD 20. Because the fetal hormonal environment (and thus fetal prostate development) is influenced by intrauterine position, male fetuses were characterized as 2F (positioned between two females), 2M (positioned between two males), or 1M (positioned between one male and one female). Only 2F and 2M males were analyzed in this study. Shortly after delivery, fetuses were sacrificed and the entire urogenital

complex was dissected. Serial sections of the urogenital complex were analyzed using computer-assisted reconstruction. In 2F males, *in utero* TCDD exposure decreased the number of prostatic epithelial buds to 65% of control in the ventral region, 70% of control in the dorsal region, and 67% of control in the lateral region. This inhibition of prostatic budding resulted in decreased total area of prostatic buds in all regions. *In utero* TCDD exposure did not significantly decrease the number of prostatic buds in 2M males, suggesting that the hormonal environment *in utero* may modulate the effects of *in utero* TCDD exposure on prostate development. (Supported by NIH grants ES01332 and AG08496.)

739 PESTICIDE RISK ASSESSMENT: USE OF A JAZZERCIZE™ ACTIVITY TO DETERMINE EXTREME CASE EXPOSURE POTENTIAL OF BROADCAST INDOOR PRODUCTS

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Broadcast use of chemicals indoors may result in unintended, unavoidable or accidental human exposures. Insecticide foggers have been of concern since they theoretically result in deposition of the most available residues on treated surfaces. Availability of those deposits for contact transfer have may be measured in persons who perform a 20 min, high contact, low impact Jazzercize activity. Chlorpyrifos (CP) was used as a model. CP parent and trichloropyridinol were measured in enviro- and biological samples. Volunteers wore whole body dosimeters (WBD) or swimming suits that covered no more than 25% of their bodies. Residues were assessed using foil deposition monitors, CDFA roller, WBD, and biological monitoring. Exposure estimates differ sharply depending upon default assumptions and monitoring methods. Regulatory "equilibrium" assumes 100% dermal absorption (DA) of total deposition yields a dosage of 1.3 mg/kg. Pre-and post-exercise surface levels were 0.11 and 0.26 ug/cm^2. Biomonitoring revealed peak excretion in urine on day 2 and a dosage of < 1.9 ug/kg (0–72h). WBD consisting of socks, gloves, and union suit retained 27, 16 and 61% of the mean potential dermal dose of 13.7 mg (range 1.3–52.6 mg). Assuming 10% clothing penetration, an experimental DA of 5.7%, and a 70 kg bw, the estimated dosage is 0.3 ug/kg. WBD and biomonitoring can be concurrently used to estimate absorbed dose under experimental conditions.

740 PESTICIDE RISK ASSESSMENT: SITUATIONAL EXPOSURE MONITORING (SEM) OF SEVERAL ACTIVITIES INVOLVING ORGANOPHOSPHATE INSECTICIDES

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Default assumptions in "worse case" risk assessment may result in estimated exposures which are misleading. When this occurs risk managers may make unreasonably conservative judgements and present misleading alternatives. SEM captures biomonitoring opportunities to gauge the relative exposure of chemical use practices to establish experimental foundation for subsequent studies. Several examples illustrate the value of biomonitoring data: 1) Indoor insecticide foggers were forecast to result in mg/kg human exposures, but estimates seem to be 2- to 3- orders of magnitude too high; 2) Glasshouse rose harvesters exposed within short time intervals to freshly sprayed foliage failed to transfer measurable residue; 3) Fully-protected mixer, loader, and applicators who applied tank mixes including organophosphates encountered ug/kg exposures; 4) Contact transfer of foliar residues resulted in harvester exposures measured in ug/kg rather than mg/kg. Preliminary knowledge of the work task obtained by SEM can guide selection of a monitoring strategy for later studies.

741 EVALUATION OF BENCHMARK DOSE CRITERIA FOR ACUTE INHALATION REFERENCE EXPOSURE LEVELS

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Recently, the Benchmark Dose Workshop concluded that an effective dose using a 1% response rate (ED_{01}) was too low for the purpose of estimating reference levels (Barnes et al., 1995). This conclusion was partly based on data from developmental toxicity studies showing that quantal benchmark dose (BMD) values at the 1% response level were, on average, 29-fold below the corresponding No Observed Adverse Effect Levels (NOAELs). We compared quantal, log-normal BMD values using 1% and 5% response rates with corresponding NOAELs from studies on a number of different chemicals and a variety of acute toxicity endpoints. Developmental toxicity data were excluded from the analysis. We found that the magnitude of difference between the BMD at a 1% response rate and the NOAEL was much smaller for acute toxicity endpoints than that previously reported by other investigators for developmental toxicity endpoints. The magnitude of difference between 1% or 5% BMD values and the NOAELs was less than 2-fold, in contrast to the 29- and 5.9-fold difference reported for developmental toxicity data sets. The lower 95% confidence limits were, on average, less than 2-fold different from the maximum likelihood estimates for the ED_{01} and ED_{05}. The BMDs based on a 1% response rate for acute effects yielded results that were 1) in close proximity to the corresponding NOAEL; 2) very close to BMDs based on the 5% response rate and 3) reasonable and consistent when considering the 95% statistical confidence limits. We conclude from these results that the exclusion of the 1% response rate as an appropriate BMD for acute endpoints other than developmental toxicity, appears to be premature.

742 PERSISTANCE OF N-NITROSO COMPOUNDS IN THE ENVIRONMENT OF CANADIAN METAL FACTORIES

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N-nitroso compounds are notorious mutagens, carcinogens and teratogens that can be seriously hazardous to human health. Metalworking fluids (MWFs) are used for lubricating and cooling the cutting and grinding surfaces of metals in metalworking machines. Because a limited survey is available on MWFs used in Canada, this study was performed to assess the magnitude of the presence of N- nitroso compounds in MWFs and in ambient air of metal factories. N-Nitrosodiethanolamine (NDELA) was found to be the major contaminant in different brands of MWFs (range 0.02 to 1.28 ppm). The nitrite could not be detected in any of the MWFs, whereas the nitrate was present in a few samples (average concentration 1.7 ppm). The air samples collected by impinger trap method contained NDELA in the range from 0.044 to 0.193 ppm. The air samples collected by Thermosorb/N technique contained the volatile N-nitrosodimethylamine (NDMA) (range 0.009 to 0.076 μg/m3). NDELA was identified and later quantitated in the environmental samples by gas chromatography-mass spectrometry (GC-MS), whereas NDMA by gas-liquid chromatography with thermal evergy analyzer (GLC-TEA). This work was supported by a grant from IRSST.

743 A PROPOSED REFERENCE DOSE (RfD) FOR CHLORPYRIFOS BASED ON RED BLOOD CELL (RBC) ACETYLCHOLIN-ESTERASE (AChE) INHIBITION

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RfD values currently used by U.S. EPA to evaluate dietary and non-dietary exposures to chlorpyrifos are based on the no-observed-effect-levels (NOELs) for inhibition of plasma cholinesterase (ChE) activity in humans and include a 10 fold safety factor. These RfD values are unnecessarily conservative for the following reasons: 1) Human plasma ChE (also known as pseudo-cholinesterase) is a different enzyme than brain AChE. 2) Plasma ChE is more sensitive to inhibition by chlorpyrifos than RBC or brain AChE. 3) Human plasma ChE is not involved in cholinergic transmission, has no known physiological function and it's inhibition is widely recognized as a marker of exposure to organophosphate insecticide rather than as a toxic effect.

RBC AChE, although not involved in cholinergic transmission, provides a better surrogate for brain AChE since the two enzymes are biochemically identical. In animals treated with chlorpyrifos, RBC AChE activity has been shown to correlate better with brain and muscle AChE activities than plasma ChE activity. The existing data indicate the single and repeated exposure RfD values for chlorpyrifos would be 0.05 and 0.01 mg/kg/day, respectively, based on the RBC AChE inhibition in humans. A RfD based on RBC AChE would be still conservative as animal data have shown that the NOEL for RBC AChE inhibition is 10 fold lower than for brain AChE inhibition and cholinergic effects are not observed until brain AChE is inhibited> 70%. Furthermore, the proposed RfD values still include an additional 10 fold safety factor for intraspecies variation. These proposed RfD values are in harmony with exposure standards established by other regulatory bodies; e.g., California EPA, European Union (EU) member countries and the World Health Organization (WHO).

744 CALCULATION OF BENCHMARK CONCENTRATIONS FOR ACUTE AND CHRONIC EXPOSURE TO ETHYLENE OXIDE (EtO)

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Sponsor: M E Andersen

The benchmark dose/benchmark concentration (BMD/BMC) approach is an alternative to the NOAEL approach that incorporates all dose-response information and is sensitive to sample size. A variety of systemic, developmental, and reproductive endpoints for EtO were modeled using several different definitions of the benchmark response. For quantal endpoints, additional risk levels of 1%, 5%, 10%, and 20% were modeled. Several different approaches were used to model continuous endpoints. The measure of change for continuous endpoints was defined in terms of the standard deviation in order to consider the natural level of variability in the endpoint under consideration. For fetal weight, modeling was conducted based on mean fetal weight per litter (a continuous parameter), and based on the counts of pups per litter with abnormally low weights (a quantal parameter). BMDs calculated by the two methods differed by a factor of 2.4, in part reflecting the fact that one method is based on increased risk, while the other is based on increased effect. Several endpoints for male reproductive toxicity were modeled (sperm head abnormalities, dominant lethals, and heritable translocations). For sperm head abnormalities, spermatids formed the most sensitive exposed population of germ cells. Because most dominant lethal mutation data (e.g., from single exposure studies) were not provided in a form appropriate for modeling, modeling of this endpoint was limited to the number of females with ≥1 dead implant. The most sensitive reproductive/developmental endpoints were dominant lethals and sperm abnormalities. The most sensitive systemic endpoints in subchronic and chronic studies were histopathologies in the adrenal cortex and kidney tubules.

745 HAZARD EVALUATION OF POLYCHLORINATED AROMATICS IN HOUSATONIC RIVER SEDIMENTS

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Analytical results[2,3] on sediment samples taken along the River showed correlated increases in levels of polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs), and polychlorinated dibenzo-p-dioxins (PCDDs). The PCDD congeners found with PCBs had 4 to 8 chlorine (Cl) atoms, but those detected in the absence of PCBs were mostly 7-Cl and 8-Cl forms. PCDFs in sediments containing PCBs were more concentrated than PCDFs in PCBs tested for toxic effects in animals. Two sediment samples having high PCB concentrations had PCDFs at 231 and 1814 mg per kg PCBs, respectively, including relatively high concentrations of the more toxic isomers. According to literature reports, PCBs tested in animals had <5 mg PCDFs per kg PCBs. These results suggest that a) PCDFs, PCDDs, and PCBs found together in sediments may have entered from a common point source; and b) human health effects additional to those expected from PCBs may occur when sediments contain both PCBs and PCDFs.

[1] This report presents the analyses and conclusions of the authors; it does not necessarily reflect the policy or views of MADEP. [2] Eitzer, B. (1993), Environ. Sci. Technol. 27:1632–1637; [3] Blasland, Bouck & Lee, Inc.(1995), Housatonic River/Silver Lake MCP Supplemental Phase II Investigation for the General Electric Co., Public Information Repository, MADEP, Springfield, MA.

746 BENCHMARK CONCENTRATION (BMC) ANALYSIS OF 1,3-BUTADIENE (BD) REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

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BMCs were derived for reproductive and developmental effects seen at low BD exposure levels in mice. Fetal weight, reduced at BD inhalation exposure levels as low as 40 ppm following in utero day 6–15 exposure (6 hr/day) (Hackett et al., 1987), was fit using a log-logistic model (Allen et al., 1994). The resulting MLE_{05} was 46.9 (BMC_{05}, 27.0) for fetuses reduced below the 5th %ile of controls. Chronic exposure to BD 6 hr/day, 5 days/week (NTP, 1991), resulted in uterine atrophy at 200 and 625 ppm in females, and testicular atrophy at 625 ppm in males; ovarian atrophy occurred at BD levels as low as 6.25 ppm. Uterine and testicular atrophy data were fit with a quantal Weibull model; ovarian atrophy data were fit using a log-logistic model. MLE_{10}s (BMD_{10}s) were 173 (105), 232 (148), and 1.8 (1.2), respectively. The BMC approach provides greater flexibility over the current NOAEL approach. Relevance to human reproduction and development must be evaluated in light of the carcinogenicity, mutagenicity, and species differences in metabolism and response to BD.

747 THE OXYGENATED FUELS PROGRAM IN NORTH CAROLINA: A PUBLIC HEALTH RISK ASSESSMENT

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The Occupational and Environmental Epidemiology Section (OEES) reviewed toxicological data on carbon monoxide (CO) and methyl tertiary butyl ether (MTBE) to evaluate the public health rationale for continuing the oxygenated fuels program in North Carolina. Data from the first three years of the oxygenated fuels program was analyzed to determine if MTBE was lowering ambient CO to health protective levels during the program period. OEES also reviewed the toxicological data for MTBE which included a detailed review of several carcinogenicity studies. The findings of this review indicated that after three years in the program CO ambient levels are well below the estimated ambient CO concentration where no observable adverse health effects would be expected and therefore, should not pose any public health risks. The study also found that MTBE failed to lower ambient CO to levels protective of public health. Finally, OEES determined that MTBE was a probable human carcinogen. OEES concludes that at this time the oxygenated fuels program utilizing MTBE in North Carolina produces no significant benefit to public health and may indeed be posing an increased health risk to the public. OEES has recommended to the state that the oxygenated fuels program be discontinued in North Carolina and in October 1995, the program was suspended for the 1995–96 winter season.

748 COMPARISON OF NONCARCINOGENIC EFFECTS AND PCB BODY BURDENS IN RHESUS MONKEYS AND HUMANS: IMPLICATIONS FOR RISK ASSESSMENT

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The U.S. Environmental Protection Agency (EPA) and the Agency for Toxic Substances and Disease Registry (ATDSR), have developed noncarcinogenic health criteria for polychlorinated biphenyls (PCBs) based on studies with rhesus monkeys. In the establishment of these criteria, it has been assumed that non-human primates are appropriate animal models for the evaluation of toxic responses in humans. However, there is increasing evidence that indicates that the rhesus monkey is uniquely sensitive to PCBs and thus a poor model for PCB toxicity in humans. In this analysis, we investigated the relevance of PCB rhesus monkey studies to the evaluation of noncarcinogenic health effects in humans. PCB body burdens measured in blood sera and associated adverse health effects were compared based on data from laboratory studies of rhesus monkeys and human epidemiologic studies. These data indicate that PCBs can produce certain systemic (dermal, ocular, and nail) and reproductive effects in rhesus monkeys with body burdens significantly lower than those observed in exposed human populations. In contrast, no significant correlation between estimated PCB body burdens and dermal, ocular, nail, or reproductive effects have been reported in human populations with PCB body burdens of up to an order of magnitude higher than the those associated with frank adverse effects in rhesus monkeys. In light of these findings, we conclude that use of the rhesus monkey for the establishment of regulatory benchmarks for PCBs should be reevaluated.

749 IMPROVING ESTIMATES OF RISK FOR WORKERS EXPOSED TO CONTAMINATED SOILS AT AGRICULTURAL SITES

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Developing risk estimates for individuals exposed directly to environmental contaminants in soils under agricultural land use scenarios is particularly problematic. Both frequency and extent of exposure to site soils can vary dramatically, depending upon the land use (type of crops, etc.) and agricultural practices (e.g., crop rotation patterns and number of crops per year, mechanized versus manual harvesting, etc.). Under these circumstances, a single set, or even limited series of, default exposure assumptions is inadequate to encompass the range of possibilities that will exist at most agricultural sites.

While site-specific exposure assumptions are clearly warranted for the majority of risk assessments of agricultural soil contamination, data to support these assumptions is not readily available. In response to this need, an analysis was conducted in which farm labor statistics for specific crops were used to derive crop-specific exposure factors. An algorithm was developed to permit use of these crop-specific factors, coupled with expert input as to the agricultural practices relevant to a particular site, to derive a site-specific worker exposure estimate. A table of crop-specific exposure factors relevant to Florida agriculture is presented, along with an example risk calculation. This approach offers a relatively straightforward means to develop more accurate, site-specific estimates of risks to farm workers from soil contamination, and may be of benefit also in developing cleanup goals for sites requiring remediation.

750 PRELIMINARY DATA ANALYSIS AND DERIVATION OF AN ESTIMATED REFERENCE DOSE (RfD_e) FOR VX

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VX, an organophosphate nerve agent, has been identified as a potential contaminant at U.S. Army non-stockpile sites. Review and analysis of data were conducted for derivation of an estimated reference dose (RfD_e) to be used in risk assessment efforts at relevant sites. Inhibition of RBC acetylcholinesterase (AChE) was used as the endpoint for identifying a Lowest Adverse-Effect Level (LOAEL). A 5-day/week, 90-day subcutaneous injection study in rats indicated a LOAEL of 0.00025 mg/kg, associated with a 52–54% reduction in RBC-AChE activity at 30 days. An equivalent oral dose was estimated from the ratio of oral and subcutaneous LD_{50} values for the rat. The resulting value was adjusted to a 7-day/week protocol. Based upon preliminary analysis of these data and application of uncertainty factors for protection of sensitive populations (10), extrapolation from LOAEL to NOAEL (10), and a deficient database (3), an RfD_e of 0.004 μg VX/kg/day is proposed pending availability of additional data or analyses (Supported by the U.S. Dept. of the Army, Army Environmental Center under IAG No. 1769–1769–A1).

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751 USE OF PUBLISHED ACUTE TOXICITY DATA TO DERIVE INTERIM EMERGENCY RESPONSE PLANNING GUIDELINES (ERPGs)

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ERPGs are being developed under the auspices of an American Industrial Hygiene Association technical committee for use in evaluating the effects of accidental chemical releases on the general public. They are estimates of concentration ranges for specific chemicals above which acute exposure would be expected to lead to adverse health effects of increasing severity for ERPG-1, -2, and -3. The Department of Energy's subcommittee on Consequence Assessment and Protective Actions (SCAPA) has recently published methodology involving a hierarchy of concentration-limit parameters to derive interim ERPG-equivalent values for chemicals of interest until ERPGs are available. Since there are no published concentration limits for many chemicals, the use of acute toxicity data has been applied to the estimation of iterim ERPG values. The parameters used include lowest (lo) observed toxic (T) and lethal (L) dose (D) and concentration (C) values, as well as 50% lethal values. TD_{lo} and TC_{lo} values were used to estimate ERPG-2-equivalents and LD_{50}, LC_{50}, LD_{lo}, and LC_{lo} were used to estimate ERPG-3-equivalents. Human data was given primary consideration, and rat data was preferred over that for other species. This was based on statistical analysis of these parameters and actual ERPGs (68 to date) and hierarchy-based ERPG-equivalents for a total of 305 chemicals requiring analysis at SRS.

752 QUANTITATIVE DOSE RESPONSE ANALYSIS-APPLICATION OF THE BENCHMARK DOSE (BMD) METHOD TO DATA FROM A 90-DAY SUBCHRONIC STUDY WITH 4-NITRO-o-PHENYLENEDIAMINE (4-NOPD)

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The BMD method was used to perform a quantitative dose-response analysis of the data from a 90-day subchronic oral toxicity study with 4-NOPD which had failed to identify a no observed adverse effect level (NOAEL). The BMD analysis identified the relevant dose to be used to derive a reference toxicity value upon which to assess safety margins for this chemical. While a BMD can be estimated for any level of risk, the $BMDL_{10}$, corresponding to a 10% probability of response is considered to be a reasonable approximation of the NOAEL. Based on the increase in liver/body weight ratios observed in male rats the smallest $BMDL_{10}$ for continuous data was 11.5mg/kg/day. This compared closely with the smallest $BMDL_{10}$ from quantal data, based on the incidence of liver hypertrophy in male rats, of 12.2 mg/kg/day. Statistically significant effects in other organ systems were also modeled and a $BMDL_{10}$ of 13.7 mg/kg/day was determined based on the incidence of thyroid follicular epithelium hypertrophy, also observed in male rats.

Use of the BMD approach on the bioassay data for 4-NOPD clearly illustrates the applicability of this method to identify a dose, the $BMDL_{10}$, which approximates to a NOAEL, in the absence of an experimentally determined value. This maximizes the use of data from such studies and obviates the need for an additional study at lower doses.

753 DEVELOPMENT OF TOXICITY VALUES FOR EMERGENCY RELEASE PLANNING

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Section 112(r) of the 1990 Clean Air Act Amendments requires that facilities prepare emergency response plans that address the potential health impacts of accidental releases of hazardous chemicals on the surrounding population. For planning purposes it is necessary to develop a scheme linking specific emergency responses to the potential effects of a given chemical concentration in air. The scientific community has developed toxicity values for evaluating possible health impacts of short-term releases for only a few chemicals, most of them highly toxic. Few of the commodity chemicals and solvents that are used in high volumes at chemical plants and refineries have determined values. The authors developed a three-tier system of toxicity values (similar to American Industrial Hygiene Association Emergency Release Planning Guidelines) to identify levels for: notification (only) of the local emergency response officials; warning to shelter in place; and recommendations to evacuate the surrounding area. Development of this system involved identification of toxicity values for 11 chemicals or chemical mixtures on the basis of their acute toxicity, taking into account the potential for chronic effects to develop after a single short-term exposure. The available toxicity data were reviewed and the levels causing irritant and/or systemic effects after one-hour exposure were determined. The level at which only mild, transient effects were expected was identified. When this level exceeded the odor threshold for the chemical we included a statement that the chemical can be smelled at levels that produce no effects. We also identified levels that might produce effects but that would not be serious or life-threatening. From this information the three guideline levels were identified for use in the emergency response plans. A one-page summary of the three guideline levels and the potential acute toxic effects of each compound was prepared for distribution to local officials.

754 QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP FOR THE EFFECT OF ALCOHOLS ON PLASMA MEMBRANE INTEGRITY *IN VITRO*

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Quantitative relationships between chemical structure and biological activity have received considerable attention in the areas of medicinal chemistry and drug development. More recently quantitative structure-activity relationships (QSAR) have been used to predict potential chemical toxicity. The objective of this study was to develop a QSAR describing the potential role of hydrophobicity in membrane integrity following alcohol exposure. Alcohols are found in numerous consumer products and are used in a variety of industrial processes. High-level exposure to some alcohols has been shown to be associated with a variety of adverse health affects. It has been proposed that alcohols elicit their toxic effects through alteration of cell membrane structure and integrity. In this study, membrane integrity (lactate dehydrogenase release) of WB rat liver cells was measured following exposure to a series of alcohols, including methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol, 1-hexanol 1-heptanol, 1-octanol, 2-butanol, 2-methyl-1-propanol, and 2-methyl-2-propanol. From these data, we have derived a QSAR equation: $\log 1/C = 0.89 (\pm 0.08) \log P - 0.10 (\pm 0.12)$; $n = 11$; $s = 0.134$, for the loss of membrane integrity in these cells following acute alcohol exposure. The data indicate

that a loss of membrane integrity is positively and linearly correlated ($r^2 = 0.986$) with hydrophobicity of the alcohols. These data may be useful in predicting the potential toxicity of novel alcohols.

755 RISK ASSESSMENT OF FLAVONOIDS AS AN ENVIRONMENTAL ESTROGEN - A STUDY CASE OF QUERCETIN

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Flavonoids are constituents commonly found in plants and taken from vegetables, fruits and herbal medicines in our daily life. Recent studies demonstrated that quercetin, a flavonoid, promotes estradiol-induced tumorigenesis through elevated level of estrogen metabolite and that flavonoids also have estrogenic activity. Flavonoids have been characterized as an environmental estrogen. Among flavonoids, quercetin shows the strongest mutagenicity tested *in vitro*. Although the carcinogenicity of quercetin is controversial, i.e. there were three positive results out of 11 carcinogenicity tests in rodents, the carcinogenic study of quercetin is only available among flavonoids. Therefore, it is imperative to evaluate the possible risk of quercetin as a representative case of flavonoids. The estimated average daily intake of quercetin is about 0.4 mg/kg/day with a body weight of 60 kg. In carcinogenic study by NTP, rats were exposed to 1,000 ppm, 10,000 ppm and 40,000 ppm quercetin for 2 years. The severity of nephropathy and the incidence of renal tubular cell adenoma in male rats increased in a dose-dependent manner. From this NTP study, NOAEL for quercetin was estimated to be less than 50 mg/kg/day (corresponding to 1,000 ppm). By employing a 200-fold safety factor to compensate for inter- and intraspecies variation, the ADI can be estimated at 0.25 mg/kg/day. The average daily intake of quercetin exceeds this ADI value, suggesting that the risk of carcinogenicity is not negligible for human exposure to quercetin through our daily life.

756 RISK ASSESSMENT FOR FUMONISIN

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A quantitative assessment of the risk from dietary exposure to fumonisin from the consumption of corn was performed to state the expected degree of harm, the extent of population variability, and the range of uncertainty. Model uncertainty, or uncertainty arising from how the data is interpreted, was represented by including model selection as a component of the uncertainty. Corn consumption was evaluated using a 3-day survey of 15,368 subjects (Continuing Survey of Food Intake by Individuals, US Department of Agriculture, 1989–1991). Standard recipes were used to estimate levels of corn in individual products. Levels of fumonisin in various corn products were based on FDA surveillance data and were represented by distributions which were fit to the data; distributional form was source of model uncertainty. Models describing a dose-response relationship between dietary exposure and renal pathology were based on data collected by Voss et al, 1993;1995. Model uncertainty arose at this step from the basic dose-response relationship, the distributional form and subject of the population variability, and the possible presence of dose-independent factors. Species extrapolation was drawn by analogy from comparisons of the toxicity of alkylating agents in rats and humans. The extent of anticipated human variability was based on evaluations of the pharmacokinetic variability among human subjects following exposure to drugs in clinical trials. The results of the analyses were assimilated with a two-dimensional array of estimates of the degree of harm in an individual. As an example of a fragment of the output, a reduction in individual effect at the 95th population percentile and the median uncertainty percentile of 10^{-5} (on a scale of 0 to 3) would be expected to result from an imposition of a control limit of 2 ppm on fumonisin levels in corn products.

757 INTERINDIVIDUAL VARIATION UNDER THE CURRENT SYSTEM OF UNCERTAINTY FACTORS

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Interindividual variation is one source of uncertainty inherent in establishing RfDs. The protectiveness of a UF of 10 to account for interindividual variation has recently been questioned. The current system of setting RfDs takes interindividual variation into account in several ways. The 10-fold UF is a measure of variation from the median response that is, a ratio of the dose that causes effects in a typical population to a sensitive population. Assuming that the dose response curve is symmetrical, the total range in susceptibility from the most sensitive to the most resistent will be the square of the ratio of the typical to the most sensitive. Therefore, the factor of 10 is adequate when the total range of sensitivity is less than 100. The use of a NOAEL in establishing an RfD provides an additional factor which can account for interindividual variation. The ratio of the ED50 to the NOAEL is roughly equivalent to the variation from the typical to the most sensitive. The use of a UF of 10 to account for interindividual variation implies that sensitivity differences between the NOAEL and the ED50 in humans are 10 times the sensitivity differences in the animals species. Assuming a 10-fold variation between the ED50 and the NOAEL and an UF of 10 the total range of susceptibility could be as high as 10,000 and still be consistent with the current system of setting RfDs.

758 RISK ASSESSMENT APPROACHES TO DEVELOPING PROPOSED NON-CANCER CHRONIC AMBIENT INHALATION REFERENCE EXPOSURE LEVELS (RELs) FOR STYRENE (STY) AND STYRENE OXIDE (SO)

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The purpose of the study is to develop proposed RELs for STY and SO, that are protective to the human population and are in accordance with OEHHA guidelines. STY, an industrial chemical, has contributed over 32 million pounds to total nationwide air emissions (USEPA 1993). SO is used commercially and in laboratory studies. Critical endpoints and no observed (or lowest observed) adverse effect levels (NOAELs or LOAELs, respectively) were identified from epidemiologic and laboratory animal inhalation dose-response studies. Adjustments were made for discontinuous exposure, and uncertainty factors (UFs) were applied to address intra- and interspecies variability, or adjustments for the use of a LOAEL or subchronic exposure duration. For STY, alternative RELs were derived from a LOAEL for central nervous system (CNS) disturbances among a chronically exposed occupational cohort and a LOAEL for lung pathology among subchronically exposed mice. Following adjustments for discontinuous exposure and the application of UFs, a STY-REL = 35 ppb (human, UFs = 100) or 1 ppb (mouse, UFs = 10,000) was obtained. A STY-REL = 35 ppb is proposed because the human study contained good dose-response data and did not require UFs associated with interspecies and subchronic extrapolations. The proposed chronic SO-REL is based on a subchronic gestational rabbit study, wherein dose-dependent lethality, lung pathology, and resorption were observed. Dam lethality was identified as the critical endpoint, and after adjusting a NOAEL for discontinuous exposure and applying UFs (1,000), a proposed SO-REL = 4 ppb was obtained. These approaches for deriving STY- and SO-RELs are under consideration as methods to provide reliable protection for the human population against STY related CNS disturbances and SO related lethality.

759 PRELIMINARY DATA ANALYSIS AND DERIVATION OF ESTIMATED REFERENCE DOSES (RfD_e) FOR GA AND GB.

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GA and GB, organophosphate nerve agents, have been identified as potential contaminants at U.S. Army non-stockpile sites. Review and analysis of data were conducted for derivation of estimated reference doses (RfD_e) to be used in risk assessment efforts at relevant sites. Inhibition of RBC acetylcholinesterase (AChE) was used as the endpoint for identifying a Lowest Adverse-Effect Level (LOAEL). A 5-day/week, 13-week intraperitoneal injection study in rats indicated a LOAEL of 0.028 mg GA/kg, associated with a 38% reduction ($p<0.05$) in RBC-AChE activity in males in the first week of exposure. An equivalent oral dose was estimated from the ratio of oral and i.p. LD_{50} values, and adjusted to a 7-day/week protocol. A 5-day/week, 13-week oral gavage study in rats indicated a LOAEL of 0.075 mg GB/kg, associated with a 23-38% reduction ($p<0.05$) in RBC-AChE activity during weeks 1-7. Based upon preliminary analysis of these data and application of uncertainty factors for protection of sensitive populations (10), interspecies extrapolation (10), extrapolation from LOAEL to NOAEL (10), and a deficient database (3), RfD_es of 0.04 μg GA/kg/day and 0.018 μg GB/kg/day are proposed pending availability of additional data or analyses (Supported by the U.S. Dept. of the Army, Army Environmental Center under IAG No. 1769-1769-A1).

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760 PRELIMINARY DATA ANALYSIS AND DERIVATION OF AN ESTIMATED REFERENCE DOSE (RfD_e) FOR LEWISITE

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Lewisite, an organic arsenical known for its vesicant properties, has been identified as a potential contaminant at U.S. Army non-stockpile sites. Review and analysis of data were conducted for derivation of an RfD_e to be used in risk assessment efforts at relevant sites. A No-Observed-Adverse-Effect Level (NOAEL) of <0.1 mg/kg day was identified in a subchronic exposure study in rats, and a teratogenicity study in rats provided a NOAEL of 1.5 mg/kg. The most definitive data regarding the toxicity of Lewisite following long-term oral exposure come from developmental toxicity studies. In a multigeneration gavage study in rats, no effects were observed in rats given 0.6 mg/kg for 13 weeks prior to mating and during gestation, and 0.34 mg/kg for 3 weeks during lactation. For assessment of potential systemic toxicity in the adult animals, a time-weighted-average dose of 0.44 mg/kg/day was calculated. Based upon preliminary analysis of these data and application of uncertainty factors for protection of sensitive populations (10), interspecies extrapolation (10), extrapolation from subchronic to chronic exposure (10), and a deficient database (3), an RfD_e of 0.1 μg/kg/day is proposed pending availability of additional data or analyses (Supported by the U.S. Dept. of the Army, Army Environmental Center under IAG No. 1769–1769– A1).

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761 PRELIMINARY DATA ANALYSIS AND DERIVATION OF AN ESTIMATED REFERENCE DOSE (RfD_e) FOR SULFUR MUSTARD (HD)

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HD, a mustard agent known for its vesicant properties, has been identified as a potential contaminant at U.S. Army non-stockpile sites. Review and analysis of data were conducted for derivation of an RfD_e to be used in risk assessment efforts at relevant sites. Epithelial hyperplasia of the forestomach was used as the endpoint for identifying a Lowest-Observed-Adverse-Effect Level (LOAEL). A 5 day/week, 13-week gavage study in rats provided a LOAEL of 0.3 mg/kg/day and a developmental toxicity study in rats provided a LOAEL of 0.4 mg/kg/day. The most definitive data, however, regarding the toxicity of HD following oral exposure came from a two-generation reproductive toxicity study in rats. A LOAEL based on epithelial hyperplasia of the forestomach was identified in rats given 0.03 mg/kg HD by gavage for 13 weeks prior to mating, during a 2-week mating period, and during gestation and lactation. Based upon preliminary analysis of these data and application of uncertainty factors for protection of sensitive populations (10), interspecies extrapolation (10), for estimating the NOAEL from the LOAEL (10), extrapolation from subchronic to chronic exposure (3), and a deficient database (3), and an RfD_e for HD of 0.0024 μg/kg/day is proposed pending availability of additional data or analyses. (Supported by the U.S. Dept. of the Army, Army Environmental Center under IAG No. 1769-1769-A1).

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762 BENCHMARK APPROACHES TO RISK ASSESSMENT OF ACETYLCHOLINESTERASE (AChE) INHIBITORS

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Benchmark methods have been receiving increased attention for estimating risks of chemical exposures. Application of the methods to risk assessment of AChE inhibitors is complicated, however, by lack of consensus on the significance of various indicators of effect. Plasma, red blood cell and brain cholinesterase activities, and clinical signs of AChE inhibition have variable relationships with dose of the inhibitors. We recommended earlier (Toxicologist 13:1640, 1993) that inhibition of all cholinesterases be considered in risk assessment, with the specific weighting of each enzyme type to be considered separately for each chemical. Here we examine proposed alternative benchmark methods for risk assessment of cholinesterase inhibitor pesticides, and compare the results to values derived using the NOEL plus an uncertainty factor. Methods are investigated for incorporation of multiple parameters (e.g., different cholinesterases, clinical signs, species, duration of exposures) into the risk estimates. The relationship of clinical signs of cholinesterase poisoning to the other measures is specifically addressed, using quantal probability distribution measures where possible. Such approaches can improve risk assessment of cholinesterase inhibitors by allowing better use of all the data, and decrease the uncertainty in extrapolation of potency estimates to humans.

763 A QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP DESCRIBING ALTERATION OF PLASMA MEMBRANE INTEGRITY BY A SERIES OF HYDROPHOBIC PHENOLS

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Human exposure to phenols is ubiquitous due to their widespread use in industry and consumer products as well as their involvement in a host of biological processes. Many phenols, such as a-tocopherol and BHT, are antioxidants, functioning as free radical scavengers. High-level exposure to some phenols has been shown to be associated with a variety of adverse health effects. The objective of this study was to determine the effects of a variety of phenols on cell membrane integrity and develop a Quantitative Structure Activity Relationship (QSAR) describing these effects. In this study, *in vitro* lactate dehydrogenase release was measured in WB rat liver epithelial cells following a one hour exposure to a series of hydrophobic phenols including phenol, 4-fluorophenol, 4-chlorophenol, 4-ethylphenol, 4-*sec*-butylphenol, *o*-cresol, *m*-cresol, and *p*-cresol. From these data, we have derived a QSAR equation:

$\log 1/C = 1.103 (\pm 0.098) \log P - 0.316 (\pm 0.214); n = 8; s = 0.053)$

for the loss of membrane integrity following acute *in vitro* phenol exposure. The present data suggest that a loss of membrane integrity is positively and linearly correlated ($r^2 = 0.992$) with hydrophobicity of these phenols. We are currently studying the *in vitro* effects of some hydrophilic phenols using similar methodology. Together, these studies may be useful in elucidating the mechanisms underlying the biological effects of phenols.

764 DEVELOPMENT OF HEALTH-BASED SCREENING LEVELS FOR SURFACE CONTAMINANTS

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Small chemical wastes storage areas were cleansed for closure. The objective of this study was to develop screening levels (SLs) to ensure that the residual chemicals on the floors, walls, and the working surfaces will not pose health hazards to humans who may come in contact with them.

To reduce household chemical wastes, many County Agricultural Commissioners' Offices in California had accepted from the public and temporarily stored residual paints and unwanted or banned pesticides in storage areas of these offices. After months or years of storage, the cans and bottles of waste chemicals were transferred and disposed of. As a part of the permit conditions, the storage areas were cleaned for closure. Confirmatory wipe samples were taken from the floors, storage shelves, and workbenches. Chemical analyses indicated the presence of pesticides (e.g., chlordane, DDT, heptachlor, methoxychlor) and metals (e.g., arsenic, barium, mercury, zinc). Thus, screening levels (SLs) were developed for these surface contaminants to provide criteria for "clean closure". The exposure pathways considered were dermal absorption and oral ingestion (hand to mouth). The toxicity factors used were Reference Doses (RfDs) for noncarcinogens and cancer potency slope factors for carcinogens. Details of the methodology and calculations for SLs for these pesticides and metals are provided in this study. Example SL values are 0.053 ug/100 cm^2 for DDT and 6.3 ug/100 cm^2 for mercury. These SLs for surface contaminants are needed to protect health of the workers who will be using the past chemical storage areas.

765 PHOTOCARCINOGENICITY TESTING FOR DERMALLY APPLIED DRUGS

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Sunlight on the skin is associated with a myriad of effects including photoaging, phototoxicity, photoallergy, immune suppression, and photocarcinogenicity. Although all are effects of concern, the potential photocarcinogenicity of topical drugs is an increasing concern at the FDA. The Division of Topical

Drug Products requests photocarcinogenicity assays for most topical drugs that are (1) applied to an area of the skin that is exposed to sunlight through normal daily life, and (2) applied chronically. Compounds of particular concern include photosensitizers, irritants, dedifferentiators, immuno-suppressants and compounds that absorb UVA or UVB (although a compound need not absorb UVR to promote photocarcinogenesis). Due to the many possible mechanisms of photocarcinogenicity promotion, we have not identified any criteria based on structure or drug class that would automatically exempt a drug from photocarcinogenicity testing. The photocarcinogenicity test we generally request is the 52-week bioassay in hairless mice (hr/hr), which includes a recovery period. The assay uses a mixed exposure of solar-simulator light and drug application and identifies only the photocarcinogenicity potential of compounds, not mechanistic information. An important goal of the Agency is to identify reliable short term assays that could distinguish non-photocarcinogens from compounds of concern.

766 STANDARDIZED ELECTRONIC DATA FILE FORMAT FOR EXCHANGE OF ANIMAL TOXICOLOGY STUDIES

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This presentation will summarize the history and the current status of a project to develop and test a standard format for the electronic exchange of animal toxicology data. This file format will greatly facilitate the electronic exchange of toxicology data within industry, within the government, and between industry and the government. Development began in the late 1980's when four Federal agencies set out to develop a format for electronic presentation of chronic toxicity and oncogenicity studies. Their proposal was published for comment in 1990 as "The Submitters' Toxicological Uniform Data Information Exchange Standard", or STUDIES. In 1992 members of the American Crop Protection Association formed the Toxicology Workgroup for the Electronic Exchange of Data (A-TWEED) to follow-up the concept of the STUDIES project. In collaboration with EPA, PhARMA, and FDA, this group has refined the STUDIES proposal, tested it, broadened it to cover virtually all standard types of animal toxicology studies, and proposed it to the X-12 committee of the American National Standards Institute as a comprehensive standard for electronic exchange of animal toxicology studies. It is expected to be promulgated by that committee as a standard for trial use by early 1996.

767 USE OF STANDARDIZED NOMENCLATURES IN TOXICOLOGY BY FDA

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In an effort to decrease the time required to review regulatory submissions, the Food and Drug Administration (FDA) is building a standardized nomenclatures database (SND) that will enhance communication between the sponsors and FDA. It will also allow data to be electronically transmitted and efficiently coded, searched, and retrieved.

For toxicology, FDA is evaluating teratology and histopathology terms used to describe preclinical data. One complete set of teratology terms, Mid Atlantic Reproduction and Teratology Association, Glossary of Fetal Alterations for Studies of Developmental and Reproductive Toxicology (93) (MG93), was identified. Recent inquiries to regulated industry show MG93 to be a *de facto* standard for describing fetal alterations. An internal FDA evaluation showed an overwhelming acceptance of MG93. In this evaluation, clarity and definition of individual terms as well as overall completeness and acceptance of MG93 were considered. Terms were scored as "criteria completely fulfilled" (0), "criteria almost fulfilled but some ambiguity exists" (1), or "criteria not fulfilled" (2). The results showed that >90% of terms were rated as 0 or 1, and <6% were rated as 2. When considering overall acceptance, MG93 was found to be "essentially complete". Based on these results, FDA has decided to proceed with external peer review of the MG93 and to convert it into a database format for further evaluation.

Initial histopathology term set requirements are presently being developed through extensive interaction with FDA reviewers and scientific staff. The status of these evaluations will be discussed.

768 SUNSCREENS: IS THERE A NEED FOR ADDITIONAL TOXICITY TESTING?

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The use of sunscreen products has been highly recommended to protect against sun-induced skin damage. Currently, 21 compounds are allowed to be marketed in the United States as sunscreen agents. Twenty of these compounds were labelled as Category I agents (safe and effective) in the 1993 OTC tentative final monograph. In addition, the monographed compounds are allowed to be used in combination with other Category I agents without reference to the vehicle. Recent data submitted to the Agency and reported in the literature have raised a concern regarding the use of combination products. In some studies, lesions were reported with a combination of Category I agents which had not been previously observed with the individual agents. In other studies, the extent of the biological response of the skin and photodecomposition were highly dependent on the formulation. The Agency is currently considering how best to allay its concerns about the potential toxicities of final formulation of sunscreens.

769 SUMMARIZING THE RESULTS OF NEUROTOXICITY TESTING: DATABASE DEVELOPMENT AND CHARACTERISTICS

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In 1985 the Office of Toxic Substances of EPA promulgated the Neurotoxicity Testing Guidelines, which were subsequently revised and published in 1991 by the Office of Pesticide Programs. Under the auspices of test rules and consent agreements for industrial chemicals, and data call-ins for pesticides, companies have submitted over 150 studies that contain data collected according to the Neurotoxicity Screening Battery (NSB) guidelines (NTIS#91-154617). This presentation outlines an effort to collate and analyze non-confidential datasets. The first phase will be the construction of a database that will allow tracking of datasets. The second phase will involve detailed data entry and methodological analyses (e.g., meta-analysis). Progress to date includes a program written in Microsoft Access®. The program tracks chemicals based on the CAS#, chemical name, and EPA Chem Code number (for pesticides). Each datafile contains information from the dataset on the submitting company, report title, authors, year, basic methodologies (e.g., species, exposure, test methods), chemical structure, and a simple description of the results. Initial efforts have been limited to datasets containing NSB data (e.g., functional observational battery, motor activity and neuropathology). Additional datasets have been included from the National Toxicology Program and the USEPA's National Health Effects and Environmental Research Laboratory.

770 A COMPARISON OF EXPOSURE LIMITS FOR HAZARDOUS CHEMICALS: DIFFERENCES AMONG FIVE AGENCIES IN THE UNITED STATES

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Exposure limits assigned by five different agencies in the US for 30 mutually listed chemicals were compared on standardized basis. Comparisons were made among EPA's Reference Dose (RfD or RfC) and Cancer Unit Risk, ACGIH's Threshold Limit Value (TLV), OSHA's Permissible Exposure Limit (PEL), NIOSH's Recommended Exposure Limit (REL), and the State of California Proposition 65's No Significant Risk Level (NSRL). Standardizations were accomplished using clearly defined assumptions (Rees et al., 1992, The Toxicologist 12(1): 97). Occupational exposure limits (TLVs, PELs, and RELs) were converted to RfD units (mg/kg/day) for comparison to EPA's RfD, which was adjusted for occupational exposure conditions. For EPA classified known or probable human carcinogens, occupational exposure limits were adjusted to lifetime daily intakes for comparison to EPA and Prop 65 daily intakes associated with no excess cancer risk at a 10E-5 level. Results indicate a general disparity according to the target population a particular limit was designed to protect. With few exceptions, guidelines for the general population were usually orders of magnitude lower than occupational limits. Differences in underlying assumptions and methods of derivation may account for some of this discrepancy, but reasons for its observed magnitude are not altogether clear.

771 DEVELOPING CONSENSUS ON THE FDA STANDARDIZED NOMENCLATURES DATABASE (SND)

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To support the harmonization of the regulatory process, the Food and Drug Administration (FDA) is evaluating controlled vocabularies to address clinical, preclinical, and other regulatory needs. Standardization of scientific vocabularies will help to harmonize the review of drugs, biologics, and other products; facilitate communications among all users of submission data; clarify the submission of data developed outside the U.S. to the FDA; and facilitate development of toxicology knowledge bases. Medical, clinical laboratory, teratology, and histopathology vocabularies, and terminology to describe experimental animal characteristics and/or demographics are the first areas being evaluated.

To develop the SND, vocabularies commonly used in the U.S. and abroad have been reviewed and evaluated for possible adoption as standards. FDA is implementing a consensus-development process to evaluate the utility and user compatibility of candidate vocabularies. The Agency requests input from affected stakeholders, including regulated industry, contract research organizations and laboratories, academia, and software developers regarding the impact of standardization of vocabularies on product development practices. FDA has identified several implementation issues including the scientific accuracy and completeness of vocabularies and the symbiosis between the working scientist and the vocabulary used to describe results. The effects of implementation of standardized vocabularies on products in development, utility of historical control data, and current toxicology data management systems also are being considered. FDA's goal is to reach a consensus that standardization of vocabularies is viable, and meets data development needs for regulatory purposes.

772 THE AIR TOXICS "HOT SPOTS" CANCER POTENCY FACTOR DATABASE

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A unit risk and cancer potency factor database for 115 compounds has been developed to facilitate cancer risk assessments performed in the state of California under the requirements of The Air Toxics "Hot Spots" Information Act of 1987 (AB2588). The compounds chosen are listed as Toxic Air Contaminants (TACs) by the California Air Resources Board (CARB). The TAC list includes the Hazardous Air Pollutants (HAPs) listed by the U.S. Environmental Protection Agency (US EPA). Unit risk and cancer potency factor factors for the compounds listed were obtained from the following sources in order of preference: TAC documents (CARB), Proposition 65 documents (Reproductive and Cancer Hazard Evaluation Section (RCHAS), Office of Environmental Health Hazard Assessment (OEHHA)), Integrated Risk Information System (IRIS) documents (Office of Health and Environmental Assessment, US EPA), Expedited Proposition 65 documents (RCHAS), Health Effects Assessment Summary Table (HEAST) entries (Office of Emergency and Remedial Response, US EPA), and OEHHA documents. A hierarchy of choice was developed for selecting cancer potency values available from multiple sources which gives preference to sources which include external peer review and public comment procedures for publishing cancer potency values. Additional weight of consideration was given to the most recent derivations using the latest data sets and scientific methodology. The database includes a summary of the key studies and methodology used to derive the unit risk factor and cancer potency factors for each compound listed. This database is undergoing external peer review (CARB Scientific Review Panel) and public comments at workshops, and is designed to be electronically accessible via both a BBS system and the Internet. The Hot Spots database will facilitate Hot Spots cancer risk assessments performed in the state of California by providing a single, easily available source for unit risk and cancer potency factors.

773 PROGRESS OF CALIFORNIA'S AIR TOXICS "HOT SPOTS" PROGRAM

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California's Air Toxics "Hot Spots" Information and Assessment Act of 1987 (AB 2588; Chapter 1252, Statutes of 1987) established a statewide program to inventory air toxics emissions from individual facilities, to assess the public health impacts of those emissions, and to notify the public of potential health risks. Staff of the 34 local air districts, the California Air Resources Board, the Office of Environmental Health Hazard Assessment (OEHHA) and facility representatives work together in implementing this statewide program. This program is among the most successful and cost-effective risk assessment review programs in existence. As of September 1995, OEHHA staff had completed the review of over 700 risk assessments from around the state. The "Hot Spots" program has been extremely effective in identifying and characterizing potentially high risk facilities. Of the facilities reviewed, approximately 83% were found to pose estimated cancer risks greater than 1×10^{-6}, and 45% of the facilities reviewed pose estimated excess cancer risks greater than 1×10^{-5}. In addition to cancer risks, many facilities reviewed have significant potential to cause adverse noncancer health impacts, such as respiratory tract irritation or impaired fertility. More comprehensive reporting of toxic emissions occurs under the "Hot Spots" program than any other program in the country. The emissions and health assessment data generated through this program provide invaluable information to regulatory programs, facilities, and the public. These data allow government and industry to prioritize risk reduction efforts towards facilities posing the greatest risks, and towards the highest risks for each individual facility. Furthermore, the "Hot Spots" program has resulted in voluntary reductions in air toxics emissions from facilities throughout the state. This presentation will focus on the implementation and results of the Air Toxics "Hot Spots" program in California.

774 DEVELOPMENT OF A TRACKING DATABASE FOR CANDIDATE AGENTS UNDER PROPOSITION 65

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The Safe Drinking Water and Toxic Enforcement Act (Proposition 65, California Health and Safety Code 25249.5 et seq.) requires the Governor to publish lists of agents known to cause cancer and reproductive toxicity. One of the mechanisms by which an agent is placed on this list is a finding by the "state's qualified experts" that an agent has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity. The "state's qualified experts" are identified in the California Code of Regulations as the Developmental and Reproductive Toxicant Identification Committee and the Carcinogen Identification Committee. Proposition 65 directs the state to engage in a diligent, thorough, and continuing search for additional chemicals which evolving scientific knowledge demonstrates are subject to the Act. Here we describe the development of a systematic process for compiling and tracking possible candidate agents and for prioritizing these agents for consideration by the appropriate Identification Committee. Key elements include (1) a database to track agents suggested for consideration, (2) acquisition of information on exposure and toxicology, (3) development of a priority list of agents, grouped into high, medium, or low priority on the basis of their potential for human exposure and level of concern about developmental/reproductive or carcinogenic hazard, (4) an assignment of priorities, and (5) expert review in the final prioritization of agents. Opportunity is provided for input and review by state agencies and departments, the scientific community, and other interested parties both in the suggestion of candidate agents and the development of the priority lists.

775 MAXIMUM ALLOWABLE LEVELS FOR ETHYLENE-DIAMINE TETRAACETIC ACID (EDTA) AND NITRILO-TRIACETIC ACID (NTA) IN POTABLE WATER

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Direct additive use of EDTA and incidental exposure to NTA (an EDTA impurity) were assessed by NSF International under ANSI/NSF Standard 60: Drinking Water Treatment Chemicals -Health Effects. NSF used data

available in the published literature to establish exposure limits under this consensus standard. The NSF evaluation of the toxicological data on EDTA and NTA lead to the establishment of maximum allowable level (MAL) values for both chemicals that apply to exposures through potable water. In accordance with Standard 60, data from cancer bioassays conducted by the National Cancer Institute (NCI) supported the presence of EDTA potable water at concentrations of up to 10 ppb (Level I exposures). Evaluation of an acceptable subchronic study raised the allowable level to 50 ppb (Level II exposures). A multigeneration study of EDTA, teratology studies in rats, and teratology studies of ethylenediamine pentaacetic acid in mice were used to satisfy the Level III data requirements. A chronic study and the NCI bioassay were used to satisfy Level IV data requirements. The MAL (2.1 mg/L) was calculated from the NOAEL in the chronic study after correcting for exposure to EDTA from foods. The NCI cancer bioassay for NTA indicated that it can cause tumors of the urinary tract. The tumor data for in rats and mice were analyzed by the linear multistage model to determine an MAL of 5 μg/L.

776 DERIVATION OF TOXICITY VALUES FOR DERMAL EXPOSURE

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Health risks from dermal exposure to chemicals were estimated by modification of oral reference doses (RfD) for non-carcinogenic effects or slope factors (q_1^*) for carcinogenic effects. By modifying the oral toxicity values by chemical-specific gastrointestinal absorption factors, the administered doses (oral RfDs and slope factors) are transformed into absorbed doses for comparison to intake amounts determined from dermal exposures. If no chemical-specific quantitative data were available in the published literature, estimates were made using structural analogs; a scheme developed to assign quantitative values to qualitative data; or default values. When mutiple quantitative data were available, the most conservative absorption factor from the species having skin most similar to the human was selected. A computer program was developed to calculate the dermal RfD and slope factors from absorption data. The program uses two data bases for calculating dermal toxicity values. The first, updated monthly, contains oral and inhalation risk values for over 600 chemicals. The second contains the absorption factor and the reference citation for over 160 chemicals. The program compares the CAS numbers of all chemicals in both data bases. When a match is found and if oral data and absorption factors are present, the program computes the dermal RfD and slope factors, prints the calculated results, the reference citations, and the source of the oral data (IRIS or HEAST). Using this methodology, RfDs ranging from 3.00E-08 to 4.00E+00 mg/kg/day and slope factors ranging from 7.89E-03 to 3.00E+05 $(mg/kg/day)^{-1}$ have been derived for over 110 chemicals.

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777 THE IMPACT OF THE CHARM ENVIRONMENTAL RISK ASSESSMENT COMPUTER MODEL ON THE OFFSHORE CHEMICAL SUPPLY INDUSTRY IN THE NORTH SEA

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The last five years have seen significant changes to the way offshore chemicals have been regulated in the North Sea. Environmental concerns have been the driving force for the introduction of harmonized control measures to try to reduce discharges of products that have an adverse effect on the marine environment. The risk to the environment associated with the use of a new chemical by an offshore operating company has become as important a consideration as the product's performance. Central to the process by which the offshore authorities approve products for use in the North Sea is the CHARM (Chemical Hazard Assessment and Risk Management) computer model. The industry has supported this project as it sees the benefits of a system based upon evaluation of the risks, rather than the inherent hazards of the product. CHARM has also produced a more transparent system for product approval, although this will not be fully realized until it has been widely implemented. The development of CHARM has also allowed industry representatives to become more involved with the offshore authorities in the development of approaches to chemical management. Some of the changes to legislation have not been viewed so positively by the offshore chemical supply industry. Negative aspects include the perception of being regulated more severely than suppliers to the land-based industry, the lack of effort to equate the amount of testing with discharge quantities, hazard-oriented pre-screening by offshore authorities, and testing and decisions based on inappropriate use of testing protocols. Despite such issues, the offshore supply industry welcomes the opportunity to be more fully involved in legislative decisions and the benefits that they will bring to the marine environment.

778 USE OF TOXICOKINETIC AND TOXICODYNAMIC DATA TO REDUCE UNCERTAINTIES WHEN SETTING OCCUPATIONAL EXPOSURE LIMITS FOR PHARMACEUTICALS

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Occupational exposure limits for pharmaceutical active ingredients should be based on all of the available preclinical and clinical data generated during drug development, including toxicokinetic (TK) and toxicodynamic (TD) data. The framework for limit setting proposed by the International Programme on Chemical Safety (IPCS) subdivides traditional 10-fold uncertainty factors (UFs) into separate half-log default values based upon TK and TD considerations unless compound-specific data are available. We propose an extension of this method by providing more detailed guidance on the development and use of TK and TD data to maximize the use of scientific information in the limit setting process. Specific recommendations are made on how TK and TD parameters may be used to support replacement of default UFs with data-derived values. Proper application of TK and TD data reduces the uncertainties when establishing limits for specific compounds and provides better assurance that established limits are providing adequate protection. It also provides the scientific support for more cost-effective exposure control strategies. In time, experience with consistent application of TK and TD data may eventually support refinements in default UFs.

779 PROPOSED METHOD FOR SETTING MCLGs FOR DRINKING WATER CONTAMINANTS BASED ON CARCINOGENIC EFFECTS

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The method used for setting MCLGs for contaminants in drinking water is based on the weight of evidence for carcinogenicity. The policy is to set the MCLG at zero for chemicals having strong evidence of carcinogenicity via ingestion. For chemicals with limited evidence of carcinogenicity, the MCLG is generally based on non-cancer endpoints with an extra uncertainty factor of up to 10 for possible carcinogenic concern. To estimate cancer risk, the default non-threshold linearized multistage model is generally used for low dose extra-polation. EPA is revising the cancer risk assessment guidelines to reflect the latest scientific knowledge. The new method will pay more attention to **mode of action, route and level of exposure.** In the absence of data to generate a biologically based model, 3 defaults will be used: linear, margin of exposure and/or both approaches. As a result, EPA is considering to revise the method for setting MCLGs. The rationale for selecting which approach for assessing cancer risk and its application to setting MCLGs will be presented. In particular, the MCLG of zero will be debated.

780 IDENTIFICATION OF RISKS FROM INDUSTRIAL SOURCES IN A CALIFORNIA RISK-BASED ASSESSMENT PROGRAM

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The Office of Environmental Health Hazard Assessment (OEHHA) reviews risk assessments conducted under the Air Toxics Hot Spots Act. OEHHA has tracked the results of risk assessments conducted by a wide variety of industrial and commercial facilities. Cancer risks reported have ranged from below one in a million to above one in a thousand. Common carcinogenic emissions include benzene, perchloroethylene, and hexavalent chromium. Cancer risk estimates for 72 of 279 facilities which had risk estimates $\geq 10^{-5}$, were driven by hexavalent chromium emissions. Cancer risks of 39 of these 279 facilities were driven by perchloroethylene. Benzene was primarily responsible for risk estimates in 31 of these 279 facilities. Emissions of polycyclic aromatic hydrocarbons drove the risk estimates in 20 of the 279 assessments. Certain facility types are associated with high estimated cancer risks. Storage of petroleum products in bulk terminals poses cancer risks from benzene as high as one in ten thousand. Use of the solvent perchloroeth-

ylene by dry cleaners can in some instances also pose risks of one in a thousand. Small facilities such as dry cleaners may contribute higher risks than some larger facilities emitting much greater quantities of chemicals largely due to proximity to receptors, poor pollution control, and poor dispersion characteristics. In some instances, small facilities may emit relatively small quantities of toxicants that are potent carcinogens (e.g., hospital waste incinerators emitting polychlorinated dibenzodioxins, and hexavalent chromium) resulting in relatively high estimated risks. In addition to cancer risks, the risk assessments also evaluate potential for noncancer adverse public health impacts using the hazard index approach. Information obtained in this program has identified the most important airborne chemicals and sources in the state.

781 PRIORITIZATION OF SUSPECTED DEVELOPMENTAL AND REPRODUCTIVE TOXICANTS FOR LISTING UNDER PROPOSITION 65: USE OF SECONDARY SOURCES

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California's "Safe Drinking Water and Toxic Enforcement Act of 1986" (Proposition 65) requires publication of a list of agents known to the state to cause cancer or reproductive toxicity. To assist in selection of agents for detailed review and consideration, OEHHA uses an initial prioritization process which has undergone public review and comment. The vast numbers of potential candidates precludes detailed review of primary literature at early stages of the process. Therefore, prioritization relies heavily upon the use of secondary sources. The Micromedex TOMES Plus(R) System allows CD-ROM searching of databases including: HSDB, IRIS, RTECS(R), Reprotext(R), Reprotox(TM), and Shepard's Catalog of Teratogenic Agents. Other particularly useful secondary sources include: ATSDR Toxicological Profiles, IARC, WHO Environmental Health Criteria documents, and standard texts such as Chemically Induced Birth Defects by James Schardein. The prioritization process includes identifying the number of studies reporting effects on particular endpoints, evaluating the consistency and severity of effects, and gathering information on the potential for human exposure. Based upon this summarized toxicological and exposure information, agents are assigned an overall priority of high, medium, low, no identified concern, or inadequate data. In general, only high-priority agents will be subject to detailed review and hazard identification. No single secondary source has proved ideal, but combining and reconciling information from a number of sources results in evaluations which satisfy the needs of the prioritization process. Information will be presented on the problems encountered in using secondary sources, the relative usefulness of different sources, and the identification of critical primary sources from secondary sources.

782 CYTOCHROME P450 INDUCTION IN RATS AFTER PERINATAL EXPOSURE TO PCB#126, PCB#118, PCB#153 OR 2,3,4,7,8-PnCDF

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The mechanism behind reproductive effects of 2,3,7,8-TCDD and PCBs after perinatal exposure is still unresolved. Around birth sexual differentiation of hepatic cytochrome P450 expression is achieved. As P450 1A1 (CYP1A1), 1A2 and 2B1 are induced by TCDD and/or PCBs, and also metabolize steroid hormones we hypothesized that: 1) perinatal exposure to PCBs and related compounds might alter hepatic P450 expression permanently; 2) subsequent alterations in hepatic steroid hormone metabolism might be involved in reproduction disorders.

Thus, mated female rats were administered a single dose of 2–121 μg 3,3',4,4',5-PnCB (PCB#126) per kg bodyweight, 0.8-81 mg 2,3',4,4',5-PnCB/kg (PCB#118), 19–275 mg 2,2',4,4',5,5'-HxCB/kg (PCB#153) or 0.4–44 μg 2,3,4,7,8-PnCDF/kg on gestation day 1. Hepatic P450 activity of the pups was measured on postnatal day 21 (PND21) and PND112. At PND21, pups exposed to PCB#126, PCB#118 or PnCDF showed dose-related induction of hepatic EROD (CYP1A1) and MROD (CYP1A2) activities. Whereas, PROD activity (CYP2B1) was induced by PCB#153 and PCB#118. PCB#126 was the most potent congener. At PND112 slight inductions in EROD, MROD and PROD were observed in offspring exposed to PCB#126, PCB#153 or PnCDF. Sex-dependent differences in P450 were found only on PND112.

From the highest dose groups male and female pups were selected, raised and tested for reproduction capacity. This offspring showed gonadal defects and reproduction disorders. Potency of the congeners decreased as follows: PCB#126 > PnCDF > PCB#118. No reproduction effects were observed in PCB#153-exposed offspring.

783 REGULATION OF NUCLEAR AH RECEPTOR FUNCTION BY PROTEIN KINASE C

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Halogenated aromatic hydrocarbons (HAHs) including polychlorinated biphenyls and dibenzo-*p*-dioxins are potent environmental contaminants. Most members of this family evoke similar biochemical and toxic responses which are thought to be mediated through the Ah receptor (AhR), a ligand-dependent transcription factor. Transcriptional activation of the *CYP1A1* gene, one of the biochemical responses induced by HAHs, has been employed in this laboratory as a model to study the cellular and molecular functions of the AhR. Experiments illustrate that cellular PKC activity is essential for the nuclear properties of the AhR. Using 101L cells, which carry a stably integrated gene containing the human *CYP1A1* promoter and DREs upstream of the luciferase reporter gene, HAHs induce *CYP1A1* transcriptional activity in a dose-dependent fashion. Pretreatment of cells with the phorbol ester, phorbol 12-myristate 13-acetate (PMA) enhanced this response 2–3 fold, implicating a role for PKC in the transcriptional activation process. This enhancement is accounted for by induced DNA binding of the nuclear Ah receptor to the DREs as measured by gel shift assays, indicating PKC is required for the nuclear accumulation of the AhR. Depletion of PKC activity by either prolonged treatment with PMA or treatment with PKC inhibitors blocked the transcriptional activation as well as the induced nuclear AhR DNA binding activity. The result may indicate that PKC activity is required for the nuclear uptake of the AhR. However, DNA binding activity of the *in vitro* activated cytosolic AhR was not affected by the activation or depletion of PKC activity. Our results indicate that the nuclear function of the AhR is dependent upon PKC activity. (Supported by USPHS Grant CA37139)

784 STIMULATION OF PROTEIN KINASE C (PKC) ACTIVITY IN LUNG BUT NOT LIVER OF AH-RESPONSIVE MICE BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)

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Activation of intracellular serine/threonine and tyrosine kinases has previously been demonstrated as an early response to TCDD exposure in rodent liver and thymus, which are considered primary targets of this compound. We have observed prolonged cytochrome P450 induction and lung tumor promotion by TCDD in Ah responsive Swiss mice. These experiments were designed to determine if pulmonary protein kinase C (PKC) could be modulated by TCDD treatment in an Ah-receptor dependent manner. Male C57 and DBA mice were given a single dose of TCDD (50 nmoles/kg, i.g.) or oil and killed 12 hrs-1 week later. Cytosolic PKC was markedly increased in lungs of C57 mice 48 hrs after TCDD, and returned to control values at 1 week. Membrane preparations demonstrated an increase in activity at the 1 week time point, suggesting translocation of the enzyme. PKC activity in DBA lungs was progressively decreased in the membranes over the 1 week time course, to 50% of control. Further, liver PKC was not altered by TCDD treatment in either mouse. The stimulation of PKC appears to be an Ah-receptor dependent effect which occurs in lung over a relatively early time course and at a dose which is not overtly toxic but maximally inducing for the P4501a family.

785 ACUTE DOSING OF TCDD RESULTS IN INCREASED HEPATIC EXPRESSION OF MURINE RAF, MEK AND MAP KINASE

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Previous studies have demonstrated that *in vivo* administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) increases expression of the cyclin-dependent kinase $p34^{cdc2}$ (CDK1). However, little is known of the effects of TCDD on upstream signaling pathways that regulate CDK1 expression. We evaluated TCDD for its ability to affect changes in expression of RAF, MEK and MAPK. RAF regulates the mitogen-activated protein kinase (MAPK)

pathway by phosphorylating and activating MEK, the dual threonine/tyrosine recognition kinase which in turn phosphorylates and activates MAPK. C57BL/6J female mice (B6) were given IP injections of 0.25, 0.5, 1, 2, 4, or 10 μg TCDD/kg or an equal volume of the corn oil carrier; hepatic S9 was prepared at 24, 36, or 48 h. RAF, MEK and MAPK were determined by immunoblots of S9 fractions with anti-RAF, anti-MEK and anti-MAPK antibodies. TCDD dosing increased RAF (3.2-fold relative to controls), MEK (2.6-fold) and MAPK (2.5-fold) in a dose-dependent manner. These data suggest that increased expression of CDK1 by TCDD involves proliferative signaling through the MAP kinase signal transduction pathway.

786 THE ARYL HYDROCARBON RECEPTOR, BUT NOT ARNT PROTEIN IS RAPIDLY DEPLETED IN VIVO AND IN VITRO FOLLOWING EXPOSURE TO TCDD

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Western blot analysis and indirect immunofluorescence microscopy were used to evaluate the fate of the aryl hydrocarbon receptor (AhR) and Arnt protein in culture cell models exposed to 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD). In wild type murine Hepa-1c1c7 cells (WT), AhR protein was depleted by 90% after 4 hours of TCDD treatment as measured in total cell lysates. In contrast, the concentration of Arnt protein was unaffected by TCDD treatment in WT cells. A reduction in AhR but not Arnt staining was observed by immunofluorescence microscopy. AhR protein was degraded in Hepa-1 type-1 variants with a similar time course and to the same maximal level observed in WT cells (85%). The EC_{50} for AhR degradation in Hepa-1 cells was 39pM and corresponded to the EC_{50} for induction of P4501A1 protein. Murine embryonic fibroblasts (NIH-3T3), rat aortic smooth muscle cells (A-7), and murine skeletal muscle cells (C2C12) all exhibited high levels of AhR degradtion (>95%) within 2–4 hours of TCDD treatment. Arnt concentration was not affected by TCDD in these cell lines. Decreased AhR but not Arnt was also observed in tissues of rats treated with TCDD. These results show that ligand binding to the AhR results in rapid degradation of this protein in the nucleus of hepatic and non-hepatic cells within the first 2–4 hours of exposure and is independent of the initial concentration of AhR or Arnt in the cell.

787 THE Ah RECEPTOR (AHR) PARTICIPATES IN THE ELECTROPHILE RESPONSE ELEMENT (EpRE)-BINDING TRANSCRIPTION COMPLEX

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The mouse dioxin-inducible [*Ah*] gene battery contains at least six genes: two Phase I cytochrome P450 genes, ***Cyp1a1*** and ***Cyp1a2***, and four Phase II genes: ***Nmo1***, NAD(P)H:menadione oxidoreductase; ***Ahd4***, a Class 3 cytosolic aldehyde dehydrogenase; ***Ugt1*06***, a UDP glucuronosyltransferase; and ***Gsta1***, glutathione transferase Ya. All six genes are up-regulated by AHR-dependent induction by dioxin involving aromatic hydrocarbon response element (AhREs), found in 5' flanking regions of all six genes. The four [*Ah*] Phase II genes are up-regulated by a Chr 7-mediated pathway acting through DNA motifs called electrophile response elements (EpREs), found in all [*Ah*] Phase II genes. Untreated ***14CoS/14CoS*** mice (also cell cultures) have a homozygous deletion of 3800 kb on Chr 7, resulting in an oxidative stress response and induction of all four [*Ah*] Phase II genes, as compared with wild-type ***ch/ch***. Using gel mobility shift assays and a synthetic EpRE oligo probe, competition with excesses of EpRE, mutated EpRE, AP-1, AhRE3, mutated AhRE3, and C/EBPα, and supershift assays with antibodies to AHR and Ah receptor nuclear translocator (ARNT), we show that the AHR (or AHR-related protein), but not ARNT, participates in the EpRE-binding transcriptional complex. ***Supported in part by NIH Grants R01 AG09235, R01 ES06811, R01 ES06273, and P30 ES06096.***

788 SOIL AND SEDIMENT DIOXIN ANALYTICAL VARIABILITY AT RISK-BASED CONCENTRATIONS

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This study examines site-specific, dioxin analytical variabilities in environmental samples to determine the technological feasibility of measuring 2,3,7,8-TCDD Toxic Equivalents (TEQs) at the Mississippi Department of Environmental Protection (MSDEQ) risk-based soil and sediment cleanup level of 4.7 parts per trillion (ppt). The potentially large economic impact of this cleanup level, in terms of both analytical and remediation costs, underscores the importance of site-specific analytical reliability measurements of the data used to make remediation or "No Further Action" decisions. Six samples, five sediment and one surface soil, were collected at 0 and 12 inches from the surface horizon; split into triplicates (a total of 18 samples); properly preserved; and submitted individually for independent laboratory analysis within 24 hours of collection. Total dioxins and furans were measured using a high resolution GC/MS (USEPA Method 8290) capable of detecting compounds in the low parts per trillion range. Total Organic Carbon (TOC) and grain size analysis were also performed. Laboratory performance and the analytical data were subjected to independent review prior to analysis. Individual sample TEQs ranged from 1.0 to 30.5 ppt with triplicate sample group arithmetic means ranging from 2.1 to 25.4 ppt. TOC ranged from 3,000 to 13,000 mg/kg and a good correlation between TOC and TEQs was observed. Intrasample TEQs variability increased dramatically at concentrations below 5 ppt and did not stabilize until mean TEQs concentrations between 15 and 20 ppt were achieved. This study demonstrates the considerable uncertainty in measuring soil and sediment TEQs at risk-based levels suggesting that such cleanup levels are neither realistic nor cost-effective because reproducible measurements are not yet technologically feasible.

789 *IN-OVO* EXPOSURE TO 2,3,7,8-TCDD RESULTS IN DELAYED EGG PRODUCTION AND DECREASED SEMINAL QUALITY

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We have previously demonstrated that chronic exposure of reproductively active White Leghorn chickens to 2,3,7,8-TCDD resulted in reproductive and endocrine effects. The dose levels of 0.01, and 2 ug/kg/day reflect the soil concentrations at Times Beach, MO before and after remediation. We reported that birds exposed to the high dose became infertile with marked changes in gonadal weight and in plasma estrogen and testosterone levels. Birds exposed to the low dose continued to produce fertile eggs but exhibited alterations in plasma estrogen and testosterone concentrations. In this study, we examined the ability of chicks (F_1 low) hatched from the low dose group to achieve normal reproduction as compared to the chicks (F_1 control) hatched from the control group through the use of a four way breeding study. F_1 low adult females began egg production approximately two weeks later than did F_1 control females. By week eight the production of eggs was equivalent. Semen quality was measured in both F_1 low adult males and F_1 control adult males. However, over 90% fertitlty was observed in all four pairing groups indicating that the reduction in semen quality was insufficient to alter reproductive success. Further studies should be done in this area to determine if domestic chicken can be used as a reproductive model for other precocial species. A special thanks to USFWS & NIEHS ES04696 for funding this research.

790 BACTERIALLY EXPRESSED GST-AhR and GST-ARNT FUSION PROTEINS SPECIFICALLY BIND DNA

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The mouse Ah receptor (AhR) and human Ah receptor nuclear translocator protein (ARNT) cDNAs were tagged with estrogen receptor epitopes and expressed as glutathione S-transferase (GST) fusion proteins in bacterial cells. Based on GST enzyme activity and western analyses, expression of both proteins was approximately 1.5-2.5 mg/l of culture. Partially purified GST-AhR did not exhibit ligand binding in hydroxyapatite and sucrose density gradient assays, alone or following co-incubation with AhR-deficient Hepa 1c1c7 C12 cytosol. However, the co-incubation induced the formation of a ligand-independent protien:DRE complex in gel retardation assays. The intensity of the specific band was significantly diminished following the addition of a 2-fold excess of unlabeled DRE probe while a 10-fold excess of unlabeled mutant DRE probe had no significant effect on band intensity. Formation of the complex was also abolished following co-incubation with a GST antibody. GST-ARNT co-incubated with ARNT-deficient Hepa 1c1c7 C4 cytosol resulted in the formation of a TCDD-inducible protein:DNA complex in gel retardation assays. This complex was effectively abolished

following co-incubation with unlabeled DRE probe while unlabeled mutant DRE had no significant effect. The TCDD-induced complex was supershifted by the GST antibody. Co-incubation of GST-AhR and GST-ARNT also resulted in the formation of a TCDD-independent protein:DNA complex that exhibited the same specificity in unlabeled probe competition assays and was supershifted by GST antibodies. These studies indicate that partially purified, bacterial expressed GST-AhR and GST-ARNT are sufficient for binding to a radiolabeled DRE probe.

791 MOUSE REGULATORY PROTEINS RECOGNIZE TROUT *CYP1A1* DNA MOTIFS CONTROLLING GENE EXPRESSION

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The trout ***CYP1A1*** gene is inducible by TCDD and other aromatic hydrocarbons. CYP1A1 induction, as described in mammalian systems, requires the binding of TCDD to the aromatic hydrocarbon receptor (AHR), dimerization with the AHR nuclear transporter (ARNT), and binding of this heterodimer to aromatic hydrocarbon responsive elements (AhREs) upstream of the ***CYP1A1*** gene. We have sequenced and characterized 1,864 bp of the 5' flanking region of the trout ***CYP1A1*** gene which contains 5 putative AhREs and many other potential transcription factor binding sites. This region was cloned into a luciferase reporter plasmid and transiently transfected into mouse hepatoma Hepa-1 cells. This construct produced 2.3-fold TCDD-inducible luciferase activity, significantly less than comparable human and mouse constructs (each 9.5-fold). Inclusion of the noncoding first intron and exon (which contains a putative AhRE) in the trout construct increases the uninduced luciferase activity and the inducibility (4.0-fold). Removal of sequences containing the AhREs eliminates TCDD-inducibility. Further deletion analysis has revealed other distinct functional domains including a strong promoter and a negative regulatory element upstream of AhRE1. Transfection of these trout constructs into mutant Hepa-1 cells deficient in AHR or ARNT has confirmed that these factors are required for transcriptional activation by TCDD. (Supported by NIH Grants RO1 ESO6811, and P30 ESO6096.)

792 SELECTIVE INHIBITION OF Ah RECEPTOR LIGAND BINDING BY SODIUM ARSENITE

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The Ah receptor (AhR) is a ligand-dependent transcription factor that binds to its DNA sequence recognition site with its heteromeric partner, Arnt. In order to define the domains and/or amino acids important in AhR functionality (ligand binding, transformation, dimerization, DNA binding and transcriptional activation) we are using various amino acid-specific chemical modification reagents. Here we describe the effects of arsenate and arsenite, two sulfhydryl modifying agents. Incubation of guinea or rat hepatic or human (LS180) cell cytosol with arsenite, but not arsenate, prior to the addition of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) results in inhibition of AhR DNA binding as assessed by gel retardation analysis. Addition of arsenite after TCDD had no significant effect on AhR DNA binding rat and guinea pig AhR, however, it inhibited DNA binding of human AhR. The loss of AhR DNA binding activity was determined to be due to an arsenite-specific inhibition of AhR ligand binding. Since arsenite, but not arsenate, can bind to vicinal dithiol groups in proteins, and the observation that dithiothreitol (which contains vicinal dithiols) can block the arsenite effect, strongly support a role for vicinal dithiols in AhR ligand binding. Not suprisingly, a region containing vicinal dithiols (3 cysteines) is present near the ligand binding domain within the rat, mouse and human AhR proteins. Our results imply that vicinal dithiols play a critical role in AhR ligand binding function, either directly or indirectly. Supported by NCI (Canada) and NIEHS (ES04699).

793 SEQUENCE COMPARISON OF PROMOTER AND ENHANCER REGIONS OF THE PYRUVATE CARBOXYLASE GENE FROM C57BL/6J($Ah^{b/b}$) AND CONGENIC($Ah^{d/d}$) MICE

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The congenic C57BL/6J($Ah^{d/d}$) mouse strain, produced by backcross/intercross breeding C57BL/6J($Ah^{b/b}$) mice with B6N· D2N(Ah^{d})N13F13 mice, has an arylhydrocarbon receptor(AhR) with a low affinity for 2,3,7,8-tetrachlorodibenzo-p-dioxin(TCDD) when compared to C57BL/6J($Ah^{b/b}$) mice. Using these strains of mice, We previously have reported that TCDD exposure reduced pyruvate carboxylase(PC) amount, activity and mRNA levels in a manner consistent with AhR involvement; i.e. $Ah^{d/d}$ mice require an approximately 60 fold higher dose necessary to produce to the same effect. It is conceivable that the differential TCDD response observed in $Ah^{d/d}$ mice relative to $Ah^{b/b}$ mice is the result of the B6N·D3N PC gene locus segregating with the AhR locus. Here we would propose that the B6N·D2N PC gene dose not response to TCDD because of regulatory sequence difference in the two mouse strains PC genes. To test this hypothesis, we cloned and sequenced the 5' regulatory region of PC for C57BL/6J($Ah^{b/b}$) and C57BL/6J($Ah^{d/d}$) mice. Our data indicate that both strains have identical 5' regulatory regions. Therefore, the PC reductions previously reported are most likely mediated by the AhR. The relationship between identifiable transcription factor binding site and the TCDD effect on PC mRNA expression are discussed.

794 AH RECEPTOR MEDIATED DOWN-REGULATION OF GENE EXPRESSION OF ESTROGEN RECEPTOR IN TISSUES OF FEMALE MICE

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2,3,7,8,-tetrachlorodibenzo-p-dioxin(TCDD) is a potent compound which is present as an ubiquitous environmental contaminant. Antiestrogenic effects are among the wide range of toxic effects reported. In order to study the mechanism of TCDD-induced suppression of estrogen receptor(ER), we quantified ER mRNA. In this study, we sought to measure the TCDD-induced decreases of ER mRNA in tissues of the response and nonresponse mice by competitive RT-PCR. The internal competitors were created with gene specific primers by PCR reaction using low annealing temperature. The relative changes of the ER mRNA of TCDD treated and control animals were determined after normalization with the level of mRNA of glyceraldehyde phosphate dehydrogenase. By using competitive RT-PCR, ER mRNA was found to be significantly suppressed in uterus, ovary and liver of female C57 BL mice (single dose 5 μg/kg, ip, four days; $p<.05, n=5$), while the suppression was not significant in the same tissues of female DBA mice by the same treatment ($p>0.05$, $n=5$). The liver *Cyp*1a1 mRNA level was greatly induced in TCDD treated C57 BL mice while no induction was seen in DBA mice. Therefore, it appears that TCDD-induced down-regulation of ER gene expression is mediated through the Ah-receptor signal transduction pathway. The work was supported in part by NIEHS Center Grant #ES 05002.

795 MOLECULAR CHARACTERIZATION OF MURINE HIF-1 cDNA

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The Ah receptor (AHR) is a ligand-activated transcription factor that mediates the biological effects of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD). The AHR and its partner the Ah receptor nuclear translocator (ARNT) are members of the basic helix-loop-helix PAS family (bHLH-PAS). Our laboratory has recently begun a search for novel members of this family. Our underlying hypothesis is that such novel proteins may interact with the TCDD pathway and may begin to explain some of TCDD's toxic effects. Previous data generated in our laboratory and others has shown that the bHLH-PAS protein HIF-1 can dimerize with ARNT and compete with AHR for ARNT interaction. In an effort to develop a murine model to study this interaction, we characterize the murine HIF-1 cDNA. In the present study, we used information from the human HIF-1 cDNA to clone the murine homologue. In this effort, a 2511 base pair open reading frame (ORF) cDNA was obtained and characterized. Comparing the mouse and human HIF-1 at amino acid level, we observed

a 95% similarity in bHLH, 99% in PAS domain and 83% in the variable domains.

796 BIOMARKERS FOR EFFECTS OF OCCUPATIONAL EXPOSURE TO PHENOXY-HERBICIDES CONTAMINATED WITH TCDD

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Biomarkers that were chosen as potential predictors for sensitivity to exposure to TCDD included the levels of Ah receptor protein, induction of CYP1A1 protein, and CYP1A1 mRNA. The cohort under study contains 109 individuals and is a subset of workers from a chemical plant in Hamburg-Moorfleet operated by Boehringer-Ingelheim. One-third of these individuals demonstrated clinical manifestations of the skin lesion, chloracne. It is generally accepted that most of TCDD's effects require binding to the Ah receptor, which initiates a wide spectrum of responses, including induction of cytochrome P450's and toxic responses. We have quantitated the total cellular expression of the Ah receptor protein in human blood lymphocytes from exposed individuals involved in this epidemiological study. In addition we have measured the induction of CYP1A1 protein and mRNA following in vitro exposure to TCDD of lymphocytes from these occupationally exposed individuals. We are in the process of correlating the response determined for these biomarkers with the levels of individual congeners found in the blood. In addition we will corollate these responses to various health effects observed to validate if these biomarkers may be predictive as exposure, effect or susceptibility markers in individuals exposed to TCDD.

797 CYTOKINES (IL-1β AND TNFα) IN RELATION TO BIOCHEMICAL AND IMMUNOLOGICAL EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) IN RATS

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Previous studies in different strains of rats and mice have shown that the inhibition of gluconeogenesis as a result of reduced liver phosphoenolpyruvate carboxykinase (PEPCK) activity together with appetite suppression play critical roles in the acute toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Recent immunological studies in rats demonstrated that exposure to low doses of TCDD resulted in an early and enhanced IgG response to immunization and an enhanced delayed-type hypersensitivity (DTH) reaction. However, high doses of TCDD suppressed the DTH reaction. This study aimed at examining by northern blot analysis the involvement of cytokines (IL-1 and TNF) in mediating the above effects. A time course study (60 μg TCDD/kg) revealed that TCDD alone can cause a transient increase of mRNA levels of TNFα and IL-1β in liver. A dose-response study in which rats were treated with TCDD (1, 3, 10, 30 and 90 μg/kg) and immunized with an antigen showed that at low doses of TCDD, mRNA levels of IL-1β were moderately increased, which may be responsible for immune function stimulation. At high doses of TCDD, mRNA levels of TNFα and IL-1β levels were greatly elevated, which may contribute to or mediate acute toxicity including immune suppression and related biochemical effects. Results from these experiments suggest that TCDD-induced cytokine changes may play important roles as mediators of various effects of TCDD.

798 2, 3, 7, 8-TETRACHLORODIBENZO-p-DIOXIN: ALTERATIONS IN HEPATIC PARENCHYMAL CELL PHARMACOKINETIC PROFILES

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We have previously reported that a single, oral, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) dose (10 or 25 ug/kg) caused alterations in isolated hepatic parenchymal cellular ureogenic contents when the cells were incubated from 0-120 minutes, using fed or 24-hour fasted male rats. Therefore, further investigations were conducted using an additional TCDD dose (2 ug/kg) and extension of the incubation time periods (0-180 minutes). Male Sprague Dawley rats were administered TCDD in a single oral dose of either 2, 10 or 25 ug/kg. Control animals received a single, equimolar, oral dose of corn oil/acetone (95%/5%, v/v). Seventy-two hours, post-oral administration, selected animals were fasted for 24-hours. Ninety-six hours after the initial oral dosing period, hepatic parenchymal cells were isolated from either fed or 24-hour fasted animals. Isolated hepatic parenchymal cells were incubated 0-180 minutes with representative incubation sample tubes taken every 10 minutes for ureogenic and gluconeogenic determinations. TCDD, administered at the lower or higher oral dose (2 or 25 ug/kg), when compared to the 10 ug/kg oral dose, produced a significant (2 fold) increase in cellular ureogenic contents in the 24-hour fasted animals. These alterations also demonstrated a cellular incubation time course relationship. Alterations in gluconeogenesis were not significate. Control values were not significantly different in these investigations. These studies indicate that hepatic parenchymal cell alterations of ureogenesis, as well as other undefined hepatic parenchymal cellular processes, might be involved in TCDD-induced pharmacokinetic processes. (Supported by CDC/ATSDR/MHPF #U50/CCU 304522-04).

799 TISSUE DISTRIBUTION OF [3H]2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) IN FEMALE SPRAGUE-DAWLEY RATS AFTER ACUTE EXPOSURE: SUBCELLULAR TCDD LEVELS IN HEPATIC AND PULMONARY TISSUES

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Tissue-distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related congeners is dose- and time-dependent with increasing accumulation of these compounds occuring in the liver with higher dose at the expense of other tissues. Even though hepatic sequestration of these chemicals exists, only the hepatic subcellular distribution of TCDD, octachlorodibenzo-*p*-dioxin (OCDD) and [125I]2-iodo-3,7,8-trichlorodibenzo-*p*-dioxin ([125I]TrCDD) is known. Therefore, we investigated the tissue and subcellular localization of [3H]TCDD in acutely exposed female Sprague-Dawley rats. Analysis of the tissue distribution data of [3H]TCDD indicates that the highest concentrations of [3H]TCDD are found in the liver. However, a dose-dependent decrease in [^{3}H]TCDD levels was observed in all other tissues examined (adipose tissue, muscle, thymus, adrenals, blood, uterus, skin, kidneys, lungs and spleen). Investigation of the subcellular distribution of [3H]TCDD in the hepatic and pulmonary tissues (%dose/total tissue or %dose/g fraction) showed a dose-dependent increase in [3H]TCDD in the hepatic P9 fraction that was not observed in the pulmonary P9 fraction. Differential centrifugation of the hepatic and pulmonary S9 subcellular fractions indicates that [3H]TCDD (%dose/total tissue) accumulates within the hepatic P100 fraction and pulmonary S100 fraction. Examination of the P100/S100 ratios of the concentration of [^{3}H]TCDD in the hepatic and pulmonary subcellular fractions indicates that [^{3}H]TCDD predominantly associates with the hepatic P100 fraction. This data suggests that the relative distribution of [^{3}H]TCDD differs in pulmonary and hepatic subcellular fractions (Supported by NAW, NIEHS ESO7126).

(This abstract does not necessarily represent USEPA policy.)

800 TPA AND SERUM RESPONSE ELEMENTS IN THE REGULATORY REGION OF THE *FOS* AND JUN GENES MEDIATE TCDD-DEPENDENT INDUCTION OF AP-1

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Halogenated aromatic hydrocarbons, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; dioxin) produce many apparently unrelated toxic effects. In rodents, dioxin causes an elevated incidence of hepatic carcinoma and pulmonary and skin tumors and, in the skin of hairless mice, promotes tumor formation at one-hundredth the dose of the classical tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). We have shown that treatment of mouse hepatoma cells with TCDD causes an increase in the mRNA levels of the immediate-early protooncogenes c-*fos*, c-*jun*, *jun-B*, and *jun-D*, and the concomitant increase of the DNA-binding activity of the transcription factor AP-1. To analyze the mechanism of *fos/jun* activation by TCDD we have used transient expression assays of luciferase reporter constructs containing response elements for TPA (TRE), serum (SRE), cyclic AMP (CRE), and aromatic hydrocarbons (AhRE) from the *fos* and *jun* genes fused to the SV40 basal promoter. We find that inclusion of TRE and SRE motifs, but not CRE or AhRE, cause a large TCDD-dependent increase in luciferase

expression. These results suggest that TCDD may induce immediate-early gene expression by activation of at least two signal transduction pathways, involving protein kinase C and tyrosine kinase(s). [cf2]Supported by NIH Grants R01 ES06273 and P30 ES06096].

801 EFFECTS OF SIGNAL TRANSDUCTION PATHWAY IN TCDD-TRANSFORMED HUMAN CELLS IN CULTURE

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TCDD is one of the most potent carcinogens ever tested and is now known as a human carcinogen at least at high dose. Neoplastic transformation of human cells in culture with exposure to TCDD has recently been reported. In the present study, TCDD-transformed human cells were characterized with respect to signal transduction pathway. In contrast to an early response of murine cell cultures, neither parental human cells nor TCDD-transformed cells showed any change of intracellular calcium concentration within 2 min. exposure of 100 nM TCDD. When treated with 100 μM extracellular ATP, parental cells and TCDD-transformed cells showed 6-fold and 2-fold increases of intracellular calcium concentration, respectively. Parental cells and TCDD-transformed cells, treated with 300 μM histamine, showed 5-fold and 3-fold increases of intracellular calcium, respectively. While TCDD-transformed cells showed a slightly higher concentration of IP_3 than parental cells, increases of IP_3 in the transformed cells, treated with 100 μM extracellular ATP and 300 μM histamine, were 2-fold and 1.6 fold less than those of parental cells, respectively. These results suggest a possible downregulation of PLC-coupling process involved in TCDD-induced carcinogenesis of human cells. This work was in part supported by the Korean Science and Engineering Foundation, grant # 951-0709-096-1.

802 *IN -OVO* 2,3,7,8-TCDD EXPOSURE IN CHICKENS: EFFECTS ON IMMUNE FUNCTION AND CYPIAI INDUCTION

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We have previously demonstrated that chronic exposure of adult White Leghorn chickens and *in-ovo* exposed 14 day hatchlings, to 2, 3, 7, 8-TCDD results in altered immune function. Dose levels of 0.01, and 2 ug/kg/day reflect soil concentrations at Times Beach, MO before and after remediation. T cell and B cell proliferation assays were run along with EROD, MROD, BROD, and PROD. Significant differences were observed in T cell and B cell proliferation in both adults and hatchlings, respectively. Adults were also noted to exhibit significant differences in CYPIAI induction; however, little induction was seen in the hatchlings. In the present study we examined the immune function and CYPIAI induction of those same hatchlings that were allowed to grow to reproductive age (30 weeks). No significant CYPIAI induction was found in the *in-ovo* exposed adults. B cell function was, however, found to still exhibit marked immunesuppression. These findings, indicate that permanent humoral immune changes occur in *in-ovo* exposed chickens at levels which do not produce CYPIAI alterations.
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803 THE DIOXIN RESPONSIVE ELEMENT: NUCLEOTIDE SEQUENCES IMPORTANT FOR Ah RECEPTOR BINDING AND ENHANCER ACTIVITY

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The Ah receptor (AhR) is a ligand-dependent DNA binding protein that interacts with a specific DNA binding site, the dioxin responsive element (DRE), prior to activation of transcription of an adjacent gene. Sequence alignment of DRE sequence from the upstream region of responsive genes has revealed a consensus of T/GNGCGTGA/C. Previous site directed mutagenesis experiments have determined that the conserved core sequence CGTG is critical for high affinity DNA binding of the AhR. Here we have extended these studies and have utilized DNA mutagenesis, gel retardation analysis and transient transfection experiments to further define the nucleotides within and adjacent to the DRE core sequence necessary for AhR binding and transcriptional enhancer activity. In addition, analysis of the DNA binding and enhancer activity of DREs identified in the upstream region of several other genes (UDP-glucuronosyl transferase, glutathione S-transferase, quinone reductase, CYP1A2 and fish CYP1A1) were carried out. The results of these studies confirm the critical nature of nucleotides contained within the core consensus sequence for DNA binding and enhancer activity. Mutation of nucleotides immediately 3' of the core sequence reduced enhancer activity to a greater extent than their effect on AhR binding affinity, demonstrating a lack of correlation between AhR binding and enhancer activity. The reason for this discrepancy is not clear, however, it may be related to an alteration in the ability of the AhR to bend DNA and/or the ability of the AhR:DRE to obtain the conformation necessary to interact with proteins important for transcription.

804 SIZE DIFFERENCE IN THE AH RECEPTORS OF THE MOST TCDD-SUSCEPTIBLE AND THE MOST TCDD-RESISTANT RAT STRAIN

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Sponsor: P G Wells

Despite an over 1000-fold difference in sensitivity to the acute lethality of TCDD between Long-Evans (Turku AB; L-E; LD50 ca. 10 μg/kg) and Han/Wistar (Kuopio; H/W; LD50>9600μg/kg) rats, these strains display similar responsiveness to several biochemical effects of TCDD, including induction of CYP1A1-associated enzyme activities (Pohjanvirta and Tuomisto, *Pharmacol Rev* 46, 483, 1994). Since the only established mechanism of action for TCDD proceeds by AH receptor-mediated transcriptional activation, we considered it integral to further compare the structure and function of the AH receptors (AHR) from the two strains. In agreement with earlier findings, no remarkable divergence was detected in ligand binding by cytosolic AHR from either liver or lung, although the consistently 2–3 fold higher concentration in L-E rats was statistically significant. However, Western blotting with a polyclonal antibody directed at the conserved N-terminal end of the AHR revealed a notable size difference in the molecule. While the AHR of the L-E rat was similar in size (ca. 105 kDa) to that reported for other rat strains, the AHR of the H/W rat was almost as small as that of the C57BL/6 mouse (ca. 95 kDa). This finding was subsequently confirmed by photoaffinity labelling. Studies are under way to further characterize the exceptionally small AHR of the H/W rat and to elucidate the possible biological consequences of this receptor polymorphism.

805 EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ON PYRUVATE CARBOXYLASE IN HEPA1C1C7 CELLS

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Tertachlorodibenzo-p-dioxin (TCDD) has been shown to down regulate pyruvate carboxylase (PC) protein and mRNA concentrations in the C57BL/6J $Ah^{b/b}$ and its congenic $Ah^{d/d}$ strains of mice. PC protein reduction was dose and time dependant. The reduction was significant at a dose of 1 ug TCDD/kg in the $Ah^{b/b}$ mice and at 75 ug TCDD/kg in the $Ah^{d/d}$ mice suggesting the involvement of the Ah receptor in downregulation of PC by TCDD. However, the effect of TCDD on PC could be a secondary response due to hormonal signals. To preclude this as a cause for the response we performed cell culture experiments using Hepa1c1c7 cells that have competent Ah receptors. These cells were exposed to TCDD at varying dose levels. Both PC levels and PC mRNA were shown to be reduced in a dose dependant manner. This is consistent with direct involvement of the Ah receptor in PC downregulation.

806 MURINE HEPATIC p53 TUMOR SUPPRESSOR PROTEIN AND CDK INHIBITORY PROTEINS $p21^{WAF1}$ and $p27^{KIP1}$, INCREASE WITH ACUTE TCDD DOSING

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) increases the expression of the cell division control enzymes $p34^{cdc2}$ kinase (CDK1), CDK2, CDK4 and CDK5. In this study the effects of acute TCDD administration on the hepatic expression of the tumor suppressor protein p53 and CDK inhibitory proteins, $p21^{WAF1}$, $p27^{Kip1}$, were evaluated using immunobloting techniques. C57BL/6J female mice were give IP injections of 0.25, 0.5, 1, 2, 4, or 10 μg TCDD/kg or the corn oil carrier alone. An hepatic 9000 x g supernatant fraction (S9) was prepared 24, 36, or 48 h following dosing. TCDD increased S9 expression of all proteins in a dosedependent manner. Maximal increases relative to controls were 2.9-fold at 0.5 μg/mL for p53, 1.7-fold at 0.5 μg/

mL for p21^{WAF1} and 1.6-fold at 1 μg/mL for p27^{Kip1}. Hyperphosphorylation of p53 was maximal (3-fold) at 0.5 μg TCDD/mL. These results indicate that, in addition to sustained proliferative signals, TCDD produces a cascade of inhibitory signals. Moreover, the simultaneous presence of these two opposing signals may create a "conflict period" in the cell that could function in the mechanism of TCDD-induced hepatocarcinogenesis.

807 CHARACTERIZATION OF THE AhR AND ARNT IN THE CH12 LX MURINE B-CELL LYMPHOMA

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The immune system has been identified as a sensitive target for the toxic effects produced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). We have previously identified the AhR and ARNT by Northern and Western analysis in splenocytes isolated from B6C3F1 mice as well as TCDD-induced binding of the AhR nuclear complex to the dioxin-responsive enhancer (DRE). The purpose of the present studies was to characterize the CH12 LX murine B-cell lymphoma as a potential model to elucidate the mechanism by which TCDD alters B-cell function. In the present studies the AhR, approximately 91 kDa, and ARNT, approximately 86 kDa, were identified in the CH12 LX cells by Western analysis. Comparison of basal AhR and ARNT gene transcription by RT-PCR indicated that approximately 2-fold more AhR transcripts exist in mouse splenocytes as compared to the CH12 LX cells and that approximately 3.5-fold more ARNT exist in mouse splenocytes as compared to CH12 LX cells. In addition TCDD treatment of the CH12 cells resulted in a dose dependent suppression of [^{3}H]-thymidine incorporation. Functional studies are ongoing to elucidate the molecular effects of TCDD on proliferation and differentiation of the CH12 LX cell line. (These studies were supported in part by the National Institutes of Health grant ES02520 and the Pharmacological Sciences Training grant GM07392).

808 CHARACTERIZATION OF THE Ah-RESPONSIVENESS OF ISHIKAWA ENDOMETRIAL CELLS

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Incubation of Ishikawa cells with 10 nM [^{3}H]2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), [^{3}H]17β-estradiol (E2), and [^{3}H]R5020 (promegestone) resulted in formation of radiolabeled nuclear aryl hydrocarbon receptor (AhR), estrogen receptor (ER), and progesterone receptor (PR) complexes with sedimentation coefficients of 6.0S, 5.0S and 3.5S, respectively, and nuclear receptor levels of 45.1 fmol/mg, 71.7 fmol/mg, and 19.3 fmol/mg, respectively. Nuclear extracts from Ishikawa cells treated with 10 nM unlabeled TCDD, E2 and progesterone formed specifically-bound DNA-protein retarded bands in gel mobility shift assays using the [^{32}P]-labeled dioxin responsive element (DRE), estrogen responsive element (ERE), and glucocorticoid responsive element (GRE/PRE). TCDD, E2 and progesterone significantly induced (1.7-fold, 1.4-fold and 1.3-fold, respectively) formation of the DRE, ERE and GRE/PRE retarded bands compared to DMSO-treated controls. Treatment of Ishikawa cells with 10 nM TCDD significantly induced ethoxyresorufin *O*-deethylase (EROD) activity by 3.1-fold (16.8 pmol/min/mg) compared to DMSO-treated controls (5.4 pmol/min/mg) and 10 nM TCDD also caused a 2.5-fold decrease in cell growth compared to DMSO-treated controls. E2-responsiveness was determined by transfection with TCDD and E2-inducible constructs. Transfected cells treated with 10 nM TCDD or 10 nM E2 showed a 6.4- and 1.8-fold inducibility, respectively. Further studies on the functional integrity of the AhR and CYP1A1 proteins are in progress.

809 REGULATION OF ESTROGEN-INDUCED TRANSFORMING GROWTH FACTOR-α BY 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN IN HUMAN BREAST CANCER CELLS

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17β-Estradiol (E2) induces transforming growth factor-α (TGF-α) gene expression in human breast cancer cell lines, and TGF-α acts as an autocrine growth factor. Previous studies in this laboratory have demonstrated that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) inhibits TGF-α-induced cell proliferation, and the molecular biology of this interaction has been further investigated using the TGF-α-Ppst1 construct which contains the -2813 to -5 5'-promoter sequence from the TGF-α gene linked to a luciferase reporter gene. Ten nM E2 induced luciferase activity 6-fold in ZR-75 human breast cancer cells transiently cotransfected with TGF-α-Ppst1 (8 μg) and a human estrogen receptor (hER) expression plasmid (1.5 μg). In contrast, treatment of the cotransfected cells with 10 nM TCDD alone did not significantly induce luciferase activity. In cells cotreated with 10 nM TCDD plus E2, there was an approximately 50% decrease in induced luciferase activity. Parallel transient transfection studies with constructs containing the -1565 to -5 and -1140 to -5 5'-promoter sequences from the TGF-α gene showed that the inhibitory effects of TCDD on E2-induced luciferase activity were also observed with these constructs. Current studies using deletion analysis and site-directed mutagenesis are focused on identification of 5' *cis*-genomic sequences in the 5'-flanking region of the TGF-α gene which are required for the aryl hydrocarbon receptor-mediated inhibitory effects.

810 GROWTH INHIBITION OF MDA-MB-468 HUMAN BREAST CANCER CELLS BY 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD): ROLE OF TRANSFORMING GROWTH FACTOR-α

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) inhibits estrogen (E2)-induced proliferation and gene expression in estrogen receptor (ER)-positive MCF-7 and T47D human breast cancer cell lines. Several ER-negative breast cancer cell lines were screened and only MDA-MB-468 cells were TCDD-responsive. Treatment of these cells with 10 nM TCDD resulted in formation of a nuclear aryl hydrocarbon (Ah) receptor complex and induction of CYP1A1 gene expression. TCDD also caused a time- and concentration-dependent inhibition of MDA-MB-468 cell growth and this response was unique since the growth of other ER-negative cell lines was unaffected by TCDD. TCDD had only minimal effects on expression of other genes associated with cell growth and differentiation; however, there was a significant 2- to 3-fold induction of transforming growth factor-α (TGFα) mRNA and secreted protein and a 1.8-fold induction of epidermal growth factor receptor mRNA levels. Moreover, subsequent treatment of MDA-MB-468 cells with 1 to 100 ng/ml of TGFα resulted in a concentration-dependent decrease in cell proliferation with significant inhibition of cell growth observed at the lowest concentration. Thus, the antiproliferative activity of TCDD in ER-negative MDA-MB-468 cells is related to induction of TGFα, a polypeptide which is normally associated with increased proliferation of breast cancer cells lines.

811 MOLECULAR MECHANISM OF INHIBITION OF ESTROGEN-INDUCED BRAIN CREATINE KINASE EXPRESSION BY TCDD

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Brain creatine kinase B (BCK) catalyzes phosphorylation of creatine, and this enzyme is induced by 17β-estradiol (E2) in a cell-type specific manner. Previous studies have shown that the BCK promoter does not contain a classical palindromic estrogen responsive element (ERE) and deletion analysis of promoter sequences identified a region (-75/-45) required for E2-responsiveness. The effects of E2, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and E2 plus TCDD were determined in estrogen receptor (ER)-positive ZR-75 cells using 2 constructs containing 2.9 kb of the BCK promoter (LPwt) and a shorter 5'-promoter flanking sequence (-195/+5) of the BCK promoter (pwt) linked to a chloramphenicol acetyl transferase (CAT) reporter gene. CAT activity in ZR-75 cells transiently transfected with a human ER (hER) expression plasmid and LPwt and treated with 10 nM E2 was induced 9.5-fold whereas TCDD alone did not cause any induction. Similar results were obtained using the pwt plasmid; however, E2-inducibility was lower (4.1-fold). In cells cotreated with 10 nM E2 plus 10 nM TCDD, there was a 40 to 50% decrease in E2-induced activity only with LPwt; TCDD did not inhibit E2-induced activity with the shorter plasmid construct. In ZR-75 cells transiently transfected with hER and LPwt, TCDD rapidly inhibited (within 2 hours) E2-induced CAT mRNA levels (88% inhibition) using an RNase protection assay. Current studies are focused on identifying *cis*-acting genomic sequences in the BCK promoter which are responsible for the inhibitory effect of TCDD.

812 SUBSTITUTED FLAVONES AS ARYL HYDROCARBON RECEPTOR AGONISTS AND ANTAGONISTS

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The effects of structure on the activity of the following 4'-and 3',4'-substituted flavones as aryl hydrocarbon (Ah) receptor agonists were investigated: flavone, 4'-methoxy-, 4'-amino-, 4'-chloro-, 4'-bromo-, 4'-nitro-, 4'-chloro-3'-nitro-, 3'-amino-4'-hydroxy, 4,4'-dichloro-, and 4'-iodoflavone. The competitive Ah receptor binding IC_{50} values varied from 4697 to 0.79 nM for 4'-nitro- and 4'-iodoflavone, respectively, and only the haloflavones induced transformation of the rat cytosolic Ah receptor complex as determined by formation of a protein-[^{32}P]dioxin response element (DRE) retarded band in a gel mobility shift assay. The haloflavones (1 μM) were also weak inducers of CYP1A1 mRNA levels in MCF-7 human breast cancer cells and 1 μM 4,4'-dichloroflavone induced 75% of the response observed for 1 nM TCDD. In MCF-7 cells, cotreated with the substituted flavones (0.01 to 10 μM) plus 1 nM TCDD, a concentration-dependent decrease in induced ethoxyresorufin *O*-deethylase (EROD) activity (> 90%) was observed for all the flavones. However, *in vitro* incubation of the substituted flavones with TCDD-induced rat hepatic microsomes also resulted in inhibition of EROD activity by direct binding the CYP1A1 protein. The interactive effects of the 10 substituted flavones (1 μM) on CYP1A1 mRNA levels induced by TCDD showed that only 4'-iodoflavone was an Ah receptor antagonist.

813 INTERACTION OF THE ARYL HYDROCARBON (Ah) RECEPTOR AND FOS ONCOPROTEIN

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The interaction of the nuclear aryl hydrocarbon (Ah) receptor complex with c-fos oncoprotein was investigated in wild-type Ah responsive mouse Hepa 1c1c7 cells. Chloramphenicol acetyl transferase (CAT) activity was induced by 10 nM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in Hepa 1c1c7 cells transfected with an Ah-responsive plasmid containing a dioxin-responsive element (DRE) and a bacterial CAT reporter gene. In cells cotransfected with the DRE-CAT construct and a c-fos expression plasmid, there was a significant decrease (50 to 60%) in CAT activity induced by TCDD. In contrast, cotransfection of Hepa 1c1c7 cells with the DRE-CAT construct and a c-jun expression plasmid had no significant effect on CAT activity induced by TCDD. The *in vitro* interactions of the nuclear Ah receptor complex from Hepa 1c1c7 cells with purified fos oncoprotein were investigated using gel electophoretic mobility shift assays. The nuclear Ah receptor complex interacted with a [^{32}P]DRE to give a specifically-bound retarded band; after coincubation of the nuclear Ah receptor complex with fos oncoprotein plus [^{32}P]DRE, a higher molecular weight retarded band was observed and this was consistent with formation of a c-fos-Ah receptor complex which still bound to the [^{32}P]DRE. The nature of the protein-protein-DNA interaction is currently being determined in crosslinking studies.

814 INHIBITION OF c-FOS PROTOONCOGENE EXPRESSION BY TCDD USING ESTROGEN-RESPONSIVE 5'-PROMOTER CONSTRUCTS

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17β-Estradiol (E2) caused a rapid induction of c-fos protooncogene mRNA levels which were maximally induced within 1 hour after treatment of MCF-7 human breast cancer cells, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) inhibited the induced response within 2 hours. The molecular mechanism of this response was further investigated using the pFC2-BL plasmid which contains the -1400 to +41 5'-flanking sequence from the human c-fos protonocogene linked to a bacterial chloramphenicol acetyl transferase (CAT) reporter gene. In MCF-7 cells transiently transfected with the pFC2-BL plasmid and treated with 10 nM E2, there was an 18.1-fold induction of CAT activity. Ten nM TCDD alone had no significant effects on CAT activity. However, in MCF-7 cells treated with 10 nM E2 plus 1 or 10 nM TCDD and transiently transfected with the PFC2-BL plasmid, there was a 52 and 73% decrease in E2-induced CAT activity, respectively. The potential *cis*-acting genomic sites for the inhibitory effects of the nuclear aryl hydrocarbon (Ah) receptor complex may be the core binding sequences of dioxin-responsive elements (DREs) which have been identified within the 5'-region (-1400/+41) of the c-*fos* gene. The importance of these sequences are being investigated using site-directed mutagenesis and transient transfection studies with plasmids containing specific region from the 5'-flanking sequence of the c-*fos* protooncogene.

815 DECREASED ARYL-HYDROCARBON (Ah)-RESPONSIVENESS BY ICI 164,384 IN MCF-7 CELLS

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MCF-7 human breast cancer cells express a functional aryl hydrocarbon receptor (AhR) and a functional estrogen receptor (ER). Treatment with the antiestrogen, ICI 164,384, increases the rate of ER degradation, and also, unexpectedly, has an impact on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-responsiveness. Induction of ethoxyresorufin *O*-deethylase (EROD) activity by 10 nM TCDD is significantly decreased in MCF-7 cells also treated with 1 μM ICI 164,384, whereas induced EROD activity in ER-independent mouse Hepa1c1c7 cells is unaffected by ICI 164,384. Thus, ICI 164,384 does not directly interact with CYP1A1; however, the results suggest a role for the ER in regulating induction of CYP1A1 by TCDD in MCF-7 cells. Transfection of MCF-7 cells with a plasmid encoding antisense ER inhibited synthesis of ER and significantly decreased induction of chloramphenicol acetyl transferase activity by TCDD in cells transiently transfected with the Ah-responsive pRNH11c plasmid. Sucrose density gradient analysis of the nuclear [^{3}H]TCDD-AhR complex shows that cotreatment of the cells with 1 μM ICI 164, 384 resulted in a 60% decrease in formation of the nuclear AhR-complex and this was paralleled by decreased formation of a specifically-bound retarded band in a gel mobility shift assay using a [^{32}P]dioxin responsive element. Results of Northern blot analysis data suggested that ICI 164,384 had no significant effect on AhR or AhR nuclear translocator mRNA levels suggesting that ICI 164,384 exerts its effect post-transcriptionally.

816 FACTORS WHICH DETERMINE THE ARYL HYDROCARBON (Ah)-NONRESPONSIVENESS OF ADRIAMYCIN RESISTANT MCF-7 HUMAN BREAST CANCER CELLS

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Adriamycin resistant (Adr^{R}) MCF-7 human breast cancer cells are an estrogen receptor negative (ER§) cell line which has been extensively used as a model for studying the mechanism of drug-resistance. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) did not induce CYP1A1 gene expression, and this was consistent with the reported Ah-nonresponsiveness of Adr^{R} MCF-7 cells. However, treatment of cells with TCDD or [^{3}H]TCDD gave a nuclear Ah receptor complex which sedimented at 6.1S and bound to a [^{32}P]dioxin-response element in a gel mobility shift assay. TCDD did not significantly induce chloramphenicol acetyl transferase (CAT) activity in cells transiently transfected with pRNHIIc, an Ah-responsive plasmid containing 5'-flanking sequences (-1142 to +2434) from the human CYP1A1 gene. In cells cotransfected with ER, Ah receptor and Ah receptor nuclear translocator (Arnt) expression plasmids, TCDD induced CAT activity only in cells expressing ER. Reverse transcriptase-polymerase chain reaction methods were utilized for characterizing the Ah receptor and Arnt transcripts in Adr^{R} MCF-7 cells. The full length (2.6-kb) Ah receptor mRNA was observed; however, only the 1.3-kb truncated Arnt mRNA transcript was detected suggesting that the lack of Ah-responsiveness was due, in part, to expression of a mutated form of Arnt. This transcript in which the transactivation domain has been deleted is also expressed in ER§ MDA-MB-231 breast cancer cells and may be a marker for the ER§ phenotype.

817 THE INVOLVEMENT OF OXIDATIVE STRESS (OS) IN TCDD-INDUCED IMMUNOTOXICIYT IN C57BL6J MICE

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The immune system is one of the most sensitive targets for TCDD and the involvement of oxidative stress (OS) in TCDD-induced immunotoxicity in C57BL6J mice is the focus of this study. To assess the involvement of oxidative stress in the toxicity of TCDD, the protective effect of the antioxidant vitamin A and vitamin E succinate against measures of oxidative stress and immunotoxicity of TCDD was studied. Mice were treated with either

vitamin A or vitamin E succinate for eight days. Half of each of the above groups of animals received TCDD on day four. Animals were sacrificed on day one or five following TCDD treatment. One day after treatment with 50 μg TCDD/kg, vitamin A and E treatment resulted in 63 and 48% decrease in DNA-single strand breaks (SSB) in peritoneal lavage cells (PLC), respectively, relative to TCDD treated mice. Following 5 days of treatment with 50μg TCDD/kg, vitamin A and E produced 85 and 78% decrease in DNA-SSB in PLC, respectively. The above treatment protocol produced 50 and 56% decrease in the production of reactive oxygen species, respectively, as measured by the cytochrome c reduction assay and 25 and 34%, respectively, as measured by the iodonitrotetrazolium assay. The effect of the antioxidant treatment on TCDD-induced immunotoxicity as measured by the secretion of IL-1 and TNF-α by PLC is under investigation. Preliminary results showed that TCDD augmented the secretion of IL-1β and TNF-α from a macrophage cell line, J774A-1 in vitro. Correlation with in vivo data remains to be established. The modulation of secretion of IL-1 and TNF-α by the antioxidant treatment may implicate a role for OS in TCDD-induced immunotoxicity.

818 INDUCTION OF CYTOCHROME P4501A1 IN PRECISION CUT SLICES OF RAT LUNG AND KIDNEY FOLLOWING IN VITRO EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)

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Cytochrome P4501A1 (CYP1A1) induction is a sensitive Ah-receptor mediated response which may serve as a biomarker for exposure to TCDD and related compounds. Precision cut rat lung and kidney (cortical) slices were incubated in dynamic organ culture for 24 hr with medium containing 0.1 or 1.0 nM TCDD followed by up to an additional 48 hr of incubation in TCDD free medium. Lung and kidney displayed a similar mean TCDD concentration of 224 ppt (wet wt) in slices exposed to 0.1 nM TCDD. Although lung and kidney slices remained viable throughout the 72 hr incubation period, CYP1A1 activity was maximally induced by TCDD at 24 hr in lung slices and at 72 hr for kidney slices. 7-Ethoxyresorufin O-deethylase (EROD), a marker of CYP1A1 activity, ranged from 2.5 pmol/min/mg in control lung slices to 102.8 pmol/min/mg after 24 hours of exposure to 1.0 nM TCDD. Kidney EROD levels at 72 hr increased from 3.3 pmol/min/mg for control slices to 31.0 pmol/min/mg for tissue exposed to 1.0 nM TCDD. A similar induction of EROD activity, although of a smaller magnitude, was observed at 0.1 nM TCDD. Further investigation will focus on CYP1A1 protein levels and the production of CYP1A1 mRNA in response to *in vitro* exposure to TCDD in this model. Human lung and kidney may also be readily utilized in this *in vitro* model as a tool for investigation of the biological and toxicological effects of exposure to TCDD and related compounds. (Supported by NIEHS grant ES06556)

819 TIME AND DOSE RELATED INDUCTION OF CYP1A1 AND CYP1A2 IN PRECISION-CUT RAT LIVER SLICES INCUBATED IN DYNAMIC ORGAN CULTURE IN THE PRESENCE OF TCDD

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In a previous 24 hr study, precision-cut rat liver slices were validated as a useful *in vitro* model for assessing the dose-related induction of CYP1A1 and CYP1A2 in rat liver following exposure to TCDD. Further assessment of the utility of this model was accomplished by initially exposing liver slices to medium containing TCDD (0.01 nM) for 24 hr and incubating the slices up to an additional 72 hr in TCDD free medium. In TCDD exposed slices, CYP1A1 protein and its respective enzymatic activity, the O-deethylation of ethoxyresorufin (EROD), significantly increased with time over the 96 hr incubation period. The magnitude of EROD induction for 24 to 96 hr ranged from 86.9 to 2021 pmol/mg/min respectively. Under identical incubation conditions, but in the absence of TCDD, the EROD activity for the control liver slices was maintained for 96 hours at approximately 17 pmol/mg/min. Conversely, the level of CYP1A2 protein and its respective activity did not show time-dependent induction in the treated group (0.01 nM TCDD), although the protein was maintained at a higher level than in the control group (O M TCDD). The dose-response relationship at 96 hr was characterized by incubating rat liver slices for the initial 24 hr in medium containing between 0.0001 pM and 10 nM TCDD. Dramatic induction of CYP1A1 protein and EROD activity was observed for all treatment groups, including the 0.0001 pM dose, which produced about a 50-fold increase in EROD activity relative to control slices. In contrast to the earlier results obtained at 24 hr, the present study indicates that incubation of liver slices for 96 hr alters the normal regulation of CYP1A1 expression in the presence of TCDD, perhaps in part through the loss of negative regulatory factors. (Supported by NIEHS grant ES06556)

820 MULTIPATHWAY ASSESSMENT OF DIOXIN UPTAKE AND RESULTING BODY BURDEN FOR SELECTED OCCUPATIONAL EXPOSURE SCENARIOS

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An approach for calculating the exposure-related TCDD body burdens from dermal, inhalation, and incidental ingestion exposures in three different worker scenarios is presented to evaluate the important factors and potential implications of TCDD uptake in selected occupations. The three different worker exposure scenarios selected for evaluation include: 1) a trichlorophenoxy herbicide manufacturing worker (20-year exposure), 2) a contract maintenance mechanic exposed by repairing a trichlorophenol reactor after and explosion incident (6-week exposure), and 3) a trichlorophenoxy applicator handling only diluted trichlorophenoxy herbicides (20-year exposure). Reasonable occupational exposure factors and published TCDD uptake rates are used to estimate absorbed dose over time, then a simple pharmacokinetic model is used to examine the relative contribution to body burden due to dietary and occupational exposure. The modeled results for the scenarios indicate that exposure to TCDD under selected work conditions can result in a wide range of potential body burden estimates. When reasonable assumptions about worker exposure scenarios are compiled with the published literature on route-specific TCDD bioavailability, the predicted body burden vs. time estimates are relatively consistent with findings in the relevant occupational epidemiological literature. In short, this work indicates that carefully conducted exposure assessments are capable of retrospectively predicting the likely uptake of persons exposed to TCDD in the past.

821 CONCURRENT APOPTOSIS AND HEPATOCELLULAR PROLIFERATION PREVENT INCREASED FORMATION OF ENZYME-ALTERED FOCI IN F344 RATS EXPOSED TO A MIXTURE OF GROUNDWATER CONTAMINANTS

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To assess the toxicity of exposure to a mixture of 7 common groundwater contaminants at low parts per million (ppm) levels, F344 rats were treated for up to six months with a drinking water mixture containing arsenic, benzene, chloroform, chromium, lead, phenol, and trichloroethylene. Controls received deionized water. Significant increases in BrdU hepatocellular labeling were present in a unique pattern surrounding large hepatic veins (0.5–2.0 mm). This did not appear to be a regenerative response due to cytotoxicity as assessed by the absence of increased plasma enzyme activity and the absence of hepatocellular lesions. To determine if increased cell proliferation might be correlated with increased risk for carcinogenesis, F344 rats were evaluated for promotion of glutathione-S-transferase (GST-P)-positive preneoplastic foci after diethylnitrosamine initiation and partial hepatectomy using Ito's Medium Term Bioassay. These studies did not find significant differences between mixture-treated animals and controls. Immunohistochemical staining for apoptosis, using the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labelling (TUNEL) method showed apoptosis in treated animals that directly correlated to areas of increased hepatocyte proliferation. This may have been triggered as a compensatory response to the increased cell proliferation or as a protective response to remove cells with altered DNA due to chemical mixture exposure.

822 INHIBITION OF PHENOBARBITAL INDUCED HEPATIC LESION PROMOTION BY DIETARY RESTRICTION IN MALE B6C3F1 MICE

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The effect of dietary restriction on hepatic lesion promotion in phenobarbital

(PB) treated B6C3F1 male mice was investigated. Preneoplastic focal lesions were formed by DEN treatment of 35 mg/kg twice a week for 8 weeks. After the focal lesions were formed, mice were randomly placed into one of the following dose groups: 1) *ad libitum* food and water 2) *ad libitum* food and 500 mg/ml PB in the drinking water, 3) 40% dietary restriction and water and, 4) 40% dietary restriction and 500 mg/ml PB in the drinking water. Mice were sacrficed after 30 and 60 days of treatment and hepatic tumor promotion parameters such as lesions per liver, preneoplastic lesion percent volume, DNA synthesis, apoptosis and gross organ weights were investigated. The number of preneoplastic lesions per liver was increased after 30 and 60 days of treatment in *ad libitum*-PB treated mice compared to *ad libitum* food and water-treatment group. In addition, the volume occupied by preneoplastic lesions was increased in the PB-*ad libitum* group when compared to PB-dietary restricted and ad libitum food and water treatment. After 60 days of treatment dietary restriction plus or minus PB decreased the number and total volume of preneoplastic lesions. PB treatment increased DNA synthesis in normal and focal tissue, but this increased DNA synthesis was inhibited by dietary restriction. Our data indicates that in this model PB functions to enhance lesion growth, yet this function can be inhibited by dietary restriction.

823 TAMOXIFEN CAUSES SOMATIC GENE MUTATIONS, DNA ADDUCTS AND HYPERPLASIA IN THE LIVERS OF BIG BLUE™ TRANSGENIC F344 RATS

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Although tamoxifen has been shown to be hepatocarcinogenic in the rat and to exhibit genotoxic activity in rat liver (DNA adduct formation, UDS and clastogenicity), it gives negative responses in bacterial and mammalian gene mutation assays that utilize exogenous metabolic activation systems. In the present study tamoxifen was administered by gavage (20mg/kg po daily for 6 weeks) to female Big Blue™ transgenic F344 rats hemizygous for the *lacI* gene. Control animals were given the solvent tricaprylin. The chemical was withdrawn for a period of 2 weeks before the animals were sacrificed. Liver DNA was extracted and mutations at the *lacI* gene locus assayed. The spontaneous mutation frequency of the *lacI* gene was $2.4 \times 10^5 \pm 0.79$; the frequency of mutations induced by tamoxifen was $11.5 \times 10^5 \pm 2.7$ ($p<0.003$). PCNA labelling of liver parenchymal cells revealed hyperplasia in tamoxifen treated rats. DNA ^{32}P post-labelling showed a significant increase in adducts after 6 weeks administration of tamoxifen ($797.4 \pm 137.3 / 10^8$ nucleotides) compared with controls ($141 \pm 53.6/10^8$ nucleotides). The results demonstrate that, using a dosing regime that has been shown to induce **DNA** adducts, hyperplasia and hepatocarcinogenesis, tamoxifen is a gene mutagen in the rat liver.

824 EFFECTS OF FENBUCONAZOLE ON MOUSE LIVER AND RELEVANCE TO LIVER TUMORS

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A slight increase in the incidence of liver adenomas was observed in female mice after 18 months of dietary exposure to fenbuconazole. Since fenbuconazole is not genotoxic and the increase in tumors was only observed at the high dose (1300 ppm), a nongenotoxic mode of action was investigated. Groups of CD-1 mice were fed 0, 20, 60, 180 and 1300 ppm (a.i.) fenbuconazole for 1 and 4 weeks. A separate group of mice was treated with phenobarbital (1000 ppm), a nongenotoxic mouse liver tumor promoter in rodents but not man. Changes in several biological markers of the liver were observed predominately in the high dose fenbuconazole group and included: liver enlargement (50%), altered liver histopathology (hepatocellular hypertrophy), increased cell proliferation (852% after 1 week) and induction of the P450 enzyme system (CYP2B form, 77-258%). In mice treated with fenbuconazole for 4 weeks followed by control diet for 6 weeks, complete reversibility was observed with all biological parameters. Phenobarbital produced almost identical responses in mouse liver. The findings demonstrate a threshold for relevant biological markers in mouse liver. These threshold effects combined with the nongenotoxic nature of fenbuconazole should be considered when evaluating oncogenic risk to man.

825 DIFFERENT MODES OF ACTION OF DICHLOROACETATE (DCA) AND TRICHLOROACETATE (TCA) IN HEPATOCARCINOGENESIS OF THE B6C3F1 MOUSE

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Dichloroacetate (DCA) and trichloroacetate (TCA) have been identified as major by-products formed during chlorination of drinking water. Neither compound appears to induce point mutations though both are hepatocarcinogenic in rodents. In order to understand possible health risks associated with these compounds, alternative mechanisms need to be explored. Male B6C3F1 mice were treated with 2.0 gm/L DCA or TCA in their drinking water for 38 or 50 weeks respectively. Groups of 12 were then given 0, 0.02, 0.1, 0.5 or 2.0 gm/L DCA or TCA for an additional two weeks. Livers were evaluated for visible hyperplastic nodules (HN) or tumors which were stained in serial sections for *c-Jun*, BrdU or *c-Fos*. Non-involved tissue (NIT) was sectioned into a minimum of six slices and stained for PAS, H&E, and BrdU to identify altered hepatic foci (AHF) that had not been apparent upon gross examination. Both DCA and TCA were found to depress cell replication in NIT. DCA was found to selectively increase rates of cell proliferation within lesions that had increased levels of *c-Jun* expression in a dose-dependent manner. Cell replication within TCA-induced tumors was very high and appeared to be independent of continued treatment. There was no selective staining of *c-Jun* within these lesions, although *c-Fos* levels were elevated where nodules were identified within tumors. These data suggest that DCA and TCA are acting via very different mechanisms in the genesis of AHF and tumors. (Supported by AWWARF/NWRI Contract 701-92/699-516-92)

826 INDOLE-3-CARBINOL ACTS AS A PROMOTER OF HEPATOCARCINOGENESIS IN RAINBOW TROUT BUT AS AN INHIBITOR IN C57 MOUSE

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Indole-3-carbinol (I3C), found in cruciferous vegetables such as broccoli and brussels sprouts, has been shown to modulate carcinogenesis when administered in the diet with different experimental animal models. I3C is also being evaluated as a potential inhibitor for breast cancer in human clinical trials. In order to more accurately assess the risk/benefit of such studies, we are investigating the promotional mechanisms and potency of I3C. In this study we examined carcinogenesis modulating effects of I3C in rainbow trout and C57 mice. Trout were initiated with 0, 25, 50, 100, 175, or 250 ppb aflatoxin B_1 (AFB_1) as embryos and I3C was given in the diet starting 3 months later at 0, 250, 500, 750, 1000, or 1250 ppm. Fish were sampled for tumors at 11 months. Significant ($p<0.03$) promotion was observed at 1000 and 1250 ppm throughout the whole initiator dose range. Male C57 black mice were injected *ip* with 2 or 5 mg/kg diethylnitrosamine (DEN) at the age of 15 days. The experimental diet, containing 1500 ppm I3C, was started at weaning. Mice were sampled for liver tumors at six and eight months. Significant inhibition was observed with the 5 mg/kg group (at the eight month sampling time-point) with I3C diet versus control diet ($p = 0.0002$). A previous study had shown that I3C promoted AFB_1-initiated hepatocarcinogenesis in rats (Selivonchick, unpublished data). *In vitro* observations in our laboratory with trout liver slices demonstrated CYP1A inducing potency of some acid condensation products of I3C, namely diindol-3-ylmethane (I33') and indolo[3,2-b]carbazole (ICZ). We are currently investigating mechanisms by which long-term post-initiation dietary I3C can act as a tumor enhancer in trout and rat, but as an inhibitor in mice. Supported by PHS grant ESO4766.

827 LACK OF HEPATIC TUMOR-PROMOTING ABILITY BY METHYL TERTIARY BUTYL ETHER

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Methyl tertiary butyl ether (MTBE), an oxygenate added to some formulations of unleaded gasoline (UG), induced liver tumors in female mice at only the high dose (8000 ppm) following chronic exposure. MTBE induces similar biological effects to those produced by UG, including its rodent tumor profile, acute hepatic effects, uterine changes suggestive of antiestrogencity, and lack of genotoxicity. Since UG is a hepatic tumor promoter in DEN-initiated female B6C3F1 mice, we tested the hypothesis that MTBE also exhibits hepatic tumor-promoting activity. Female B6C3F1 mice were injected with a single i.p. injection of N-nitrosdiethylamine (DEN) (5mg/kg body weight) at 12 days of age. Exposure to MTBE (7988 ppm) under bioassay conditions

was begun at 8 weeks of age. After 4 months of exposure, MTBE significantly increased relative liver weight and hepatic microsomal P450 pentoxyresourfin-O-dealkylase (PROD) activity. DNA synthesis in nonfocal hepatocytes did not change after MTBE exposure as compared to controls. MTBE did not significantly increase the number or size of hepatic foci or volume fraction of the liver occupied by hepatic foci on H&E-stained sections as compared to DEN-initiated controls. Relative uterine, ovary and pituitary weights were decreased in MTBE-exposed mice as compared to controls. Although MTBE produces acute effects similar to UG, MTBE does not exhibit tumor-promoting activity in DEN-initiated B6C3F1 female mice at four months. However, MTBE produced endocrine modulations suggestive of estrogen antagonism. (supported in part by grants from the Oxygenated Fuels Association and NIEHS ES-05641-01.)

828 PROTON PUMP INHIBITOR PROTECTS AGAINST PHENOBARBITAL LIVER TUMOR PROMOTION

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Acute/subchronic liver findings in the liver of rodents exposed to dosages of drugs that result in liver tumorigenesis when exposed daily over a lifetime have potential relevance in a mechanistic sense. Robust P450 microsomal enzyme induction associated with liver hypertrophy represent the most frequent biologic effect in these short term studies (excluding estrogenic and androgenic therapeutics). Because the by-products of cell metabolism are acidic and because of the growing evidence that pH perturbation can promote carcinogenesis, we have hypothesized that the prototype drug phenobarbital may promote as a consequence of pH or sodium pump perturbation at the level of the hepatocyte.

As one of a series of activities to test the hypothesis we treated one year old male C3H/J mice with 500 ppm phenobarbital in the drinking water plus the proton pump inhibitor omeprazole for four months. Livers from these mice were compared with those from negative naive controls as well as with the positive phenobarbital only control. The livers of both groups receiving phenobarbital were hypertrophic consistent with the expected changes of robust microsomal enzyme induction. However omeprazole protected the mice from the liver tumor promoting influence of phenobarbital. Furthermore, dietary manipulation to create systemic acid/base stress with sodium bicarbonate or ammonium chloride correspondingly modulated hepatic phenobarbital promoted tumorigenesis.

829 RELATIONSHIP BETWEEN SELENIUM AND ANTIOXIDANT ENZYMES: TWO MONTH STUDY

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Selenium (Se) has been reported to exert protective effects against carcinogenesis and alteration of antioxidant enzymes is one proposed mechanism. In order to evaluate this potential mechanism, partially hepatectomized rats were pretreated with different concentrations of Se (0.0, 0.1, 0.25, 0.5, 1.0, and 2.0 ppm) and then exposed to diethylnitrosamine to induce hepatocarcinogenesis. Se treatment was continued for 60 days.

Liver microsomes and cytosolic fractions were analyzed for catalase (CAT), glutathione peroxidase (GSHPx), superoxide dismutase (SOD), as well as Se content. Lipid peroxidation was also assessed. Our data shows a direct relationship between Se provided in drinking water and measured liver concentration. It also indicates that the activities of CAT and GSHPx increase as Se concentrations in liver increase. At higher concentrations, CAT activity decreased whereas SOD activity was unaffected by Se. The rate of lipid peroxidation decreased as Se concentration increased; at the highest Se concentration, the trend reversed.

This two month data correlates well with our previous observations made after one month of Se exposure which provides some support to the proposed mechanism. Histochemical analyses are in process to correlate these findings with precarcinogenic foci formation.

830 REVERSIBILITY OF PROMOTER INDUCED HEPATIC FOCAL PRENEOPLASTIC LESION GROWTH IN MALE B6C3F1 MICE

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The effect of dietary removal of phenobarbital and dieldrin on focal lesion growth in male B6C3F1 mice initiated with diethylnitrosamine (DEN) was investigated. After preneoplastic lesions were formed, mice were randomly placed into either dieldrin (10.0 mg/kg diet), phenobarbital (500 mg/kg diet) or control NIH-07 diet dose groups and killed after 30 and 60 days of dietary treatment. The effect of treatment with dieldrin or phenobarbital upon lesion growth was examined by measuring DNA synthesis, apoptosis, the number of preneoplastic lesions per liver and the focal lesion volume percentage. After 30 and 60 days of dietary treatment, dieldrin and phenobarbital treatment resulted in increased number and volume of preneoplastic lesions. Replicative DNA synthesis in preneoplastic focal lesions was also increased. Removal of dieldrin and phenobarbital from the diet for 30 days after 30 days of promoter treatment resulted in decreased number, volume, and labeling index and increased apoptotic rate of preneoplastic lesions, when compared to mice treated with dieldrin or phenobarbital continuously for 60 days. These data suggest that induction and maintenence of preneoplastic lesion growth is dependent upon continous tumor promoter treatment.

831 TRANSCRIBED ALLELE OF IMPRINTED M6P/IGF2 RECEPTOR GENE IS MUTATED IN RAT LIVER TUMORS

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We have recently provided mutational evidence that the *M6P/IGF2r* is a tumor suppressor gene in human hepatocellular carcinomas (De Souza *et al.* Nature Genet., in press). The *M6P/IGF2r* locus is imprinted in mice but not in most humans. We determined in this investigation, with the use of a DNA sequence polymorphism in the *M6P/IGF2r* gene, that this gene is also maternally imprinted in Fischer (F344), Lewis and Brown Norway rats. In addition, using RT-PCR and direct PCR sequencing from RNA and DNA, we have found mutations in the expressed allele of the *M6P/IGF2r* in liver tumors from male F344 rats initiated with diethylnitrosamine and promoted with phenobarbital. This provides further evidence that the M6P/IGF2r is also important mechanistically in rat hepatocarcinogenesis. However, humans would be more resistant to liver tumor formation than rats and mice when the M6P/IGF2r is involved in the tumorigenic process. (Supported by NIH grant CA25951, ILSI and Procter & Gamble).

832 CORRELATION OF BASAL LEVELS OF OXIDATIVE DNA DAMAGE WITH SUSCEPTIBILITY TO LIVER TUMORIGENESIS IN INBRED STRAINS OF MICE

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Susceptibility to spontaneous or chemically induced liver tumors in mice has been demonstrated to be strain dependent, with the tumor development or promotion phase contributing most to variability. Since reactive oxygen species are thought to play a role in carcinogenesis, especially tumor promotion, we investigated steady-state levels of 8-hydroxy-2'-deoxyguanosine (8-oxo-dG) in age matched (7 months), untreated mice of three inbred strains that differ significantly in their susceptibility to liver carcinogenesis. Male mice of strain C3H, highly sensitive to liver tumorigenesis, had significantly higher levels of hepatic 8-oxo-dG (3.3 ± 0.2 8-oxo-dG/10^5 dG) as compared to those of resistant strains C57BL ($2.1 \pm 0.3/10^5$ dG; $p<0.01$) and A/JCr mice ($2.5 \pm 0.1/10^5$ dG; $p<0.015$). In contrast, levels of 8-oxo-dG in livers of female A/JCr mice ($3.1 \pm 0.3/10^5$ dG) were higher than in those of C3H ($2.2 \pm 0.1/10^5$ dG; $p<0.025$) and C57BL females ($2.5 \pm 0.3/10^5$ dG; p = NS). Male C3H livers presented significantly more 8-oxo-dG than those of C3H females ($p<0.002$). These results suggest that steady-state levels of 8-oxo-dG may contribute to the inherent differences in susceptibility to hepatocarcinogenesis in inbred strains of mice, especially the high sensitivity of C3H males.

833 EXPRESSION OF TRANSFORMING GROWTH FACTOR-α (TGFα) IN ESTABLISHED RAT HEPATIC FOCI TREATED WITH TUMOR PROMOTERS

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TGFα is a positive-growth regulator in epithelial cells. Its overexpression has been associated with increased growth and malignancy of end-stage cancers such as breast cancer, pancreatic cancer, ovarian carcinoma, and hepatocellular carcinomas in humans and rodents. The primary aim of this study was to examine the effects of tumor promoters on TGFα expression in established rat hepatic foci. Hepatic lesions were induced by two i.p. injections (one week apart) of diethylnitrosamine into 4-week-old male F344

rats, and lesions were allowed to develop for seven months before promoter treatment. Rats were sacrificed 30 days after receiving 500 ppm phenobarbital (PB) or 6000 ppm diethylhexylphthalate (DEHP) in the diet or partial hepatectomy (PH). Saline-treated controls received similar promoter treatments. One week prior to sacrifice, the rats received an osmotic minipump containing BrdU to allow measurement of DNA synthesis. Tissues were fixed in formalin, paraffin embedded, and serially cut onto glass microscope slides. Each slide was either stained with hematoxylin and eosin or antibody-labeled for TGFα or BrdU. In controls, 13.7% of all foci stained positive for TGFα compared with 53.6%, 35.3%, and 48.6% observed in DEHP, PB, and PH, respectively. When subcategorized by histological phenotype, eosinophilic foci exhibited the highest percentage of TGFα-positive foci in all treatment groups. Treatment with DEHP stimulated TGFα expression in 34.5% of basophilic foci compared with 10.5% observed in controls. Foci expressing TGFα were also likely to have high levels of DNA synthesis (>30%BrdU) regardless of treatment. However, tumor promoters stimulated the expression of TGFα in foci with intermediate levels of DNA synthesis (10-30% BrdU). These findings suggest that tumor promoters may exert some of their prolierative effect by stimulating production of TGFα.

834 EVIDENCE OF CARCINOGENIC ACTIVITY OF PENTACHLOROBENZENE BASED ON INCREASED GST-P ALTERED LIVER FOCI IN F344 RATS

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As part of an effort to assess the carcinogenic potential of pentachlorobenzene (PeCB), cell kinetics were studied in glutathione S-transferase positive (GST-P) foci induced in male Fischer 344 rats using Ito's Medium-Term Bioassay. Rats were divided into five groups. Three groups were given initiating injections (200 mg/kg ip) of diethylnitrosamine (DEN) and the remaining two groups were given saline injections. Two weeks following DEN injections, one group was given a daily gavage dose of 0.4 mmol/kg PeCB, the other group was given 0.1 mmol/kg PeCB, and the third group was gavaged with corn oil. Similarly, the two saline groups were treated with either 0.4 mmol/kg PeCB or corn oil. At week 3, a partial hepatectomy was performed and the gavage dosing of PeCB (or corn oil) continued for a total of 6 weeks. Cell proliferation and apoptosis rates within GST-P foci and surrounding normal liver were measured using proliferating cell nuclear antigen (PCNA) and *in situ* end-labeling techniques, respectively. Preliminary results indicate a significant increase in foci area in both total foci ($p = 0.0004$) and foci greater than 0.2 mm in diameter ($p = 0.0013$) in rats treated with 0.1 mmol/kg PeCB. However, the number of foci was not significantly greater in the PeCB treated rats ($p = 0.23$).

835 LACK OF HEPATIC TUMOR-PROMOTING ACTIVITY OF UNLEADED GASOLINE IN OVARIECTOMIZED MICE

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Chronic exposure to unleaded gasoline (UG) vapor at the highest dose of 2056 ppm selectively increases the incidence of liver tumors in female mice. After subchronic exposure UG exhibits tumor-promoting activity in N-nitrosdiethylamine (DEN)-initiated mouse liver. Estrogen inhibits mouse liver tumor development, and the hepatocarcinogenic and tumor-promoting doses of UG produce uterine changes suggestive of estrogen antagonism. Thus we tested the hypothesis that UG-induced tumor-promoting ability was secondary to its estrogen antagonism by comparing the tumor-promoting ability of UG in ovariectomized (ovex) mice to the hepatic tumor-promoting ability of UG in intact mice. Female B6C3F1 mice were injected with DEN (5mg/kg body weight) at 12 days of age. Ovex mice had ovaries surgically removed at 4 weeks of age. Exposure to wholly vaporized UG (2018 ppm) under bioassay conditions began at 8 weeks of age. UG increased relative liver weight and hepatic microsomal P450 pentoxyresourfin-O-dealkylase (PROD) activity to a similar extent in intact and ovex mice. Nonfocal hepatocyte proliferation, as measured by the incorporation of bromodeoxyuridine,m was not changed by UG exposure or ovariectomy. After 4 months of exposure to DEN-initiated mice, UG significantly increased the volume fraction of liver occupied by foci (three fold) as compared to controls in intact mice. In contrast, there was no significant increase in the focal volume fraction in ovex mice exposed to UG as compared to ovex control mice. These data suggest that ovarian hormones are required for the tumor-promoting activity of UG. (Supported in part by grants from the American Petroleum Institute and NIEHS ES-05641-01.)

836 TIME AND DOSE DEPENDENT PROMOTION OF ALTERED HEPATIC FOCI BY 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN

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Though not a known human carcinogen, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin(TCDD) is a prototypical rat liver tumor promoter. The objective of the current study was to investigate the dose and time dependence of TCDD mediated promotion of altered hepatic foci (AHF) in the two-stage model of rat liver carcinogenesis. Female Sprague-Dawley rats were administered a single dose of diethylnitrosamine (DEN, 10mg/kg, p.o.) 24 hours after partial hepatectomy. Animals were administered TCDD biweekly at 0.0μg/kg, 0.14μg/kg, 0.014μg/kg, 0.0014μg/kg or .00014μg/kg. Animals were sacrificed at 1,3 or 6 months, and four serial liver sections were each stained for different markers of AHF. The number and volume fraction of AHF was determined by quantitative stereology. With one month exposure to TCDD, increases in the number and volume fraction of AHF were observed for each marker at the two highest doses of TCDD in the DEN-treated animals. High dose TCDD treatment significantly increased the total number and volume fraction of AHF in the three and six month groups which also received DEN. The volume fraction of AHF increased with time in all DEN treated groups. Response to TCDD treatment was not linear within the dose range examined for both DEN and non-DEN treated groups.

837 THE UTILIZATION OF THE MEDIUM-TERM LIVER FOCI BIOASSAY FOR STUDYING THE CARCINOGENICITY OF HEXACHLOROBENZENE (HCB) IN FISCHER 344 RATS

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To study the mechanism of carcinogenesis of hexachlorobenzene (HCB) for predictive cancer modeling, we utilized the Ito's Liver foci bioassay. At the beginning of the eight-weeks protocol, 54 rats were given ip injections of an initiating dose of dimethylnitrosamine (DEN) whereas the remaining 30 animals were injected with saline. Of the DEN injected rats, 18 were daily gavaged with 0.4 mmoles/kg/day of HCB (DEN-high group), 18 rats were given 0.2 mmoles/kg/day of HCB (DEN-low group), and 18 rats were given corn oil as vehicle (DEN-veh group). Of the saline injected groups, 18 were daily gavaged with the high dose of HCB (SAL-high group), and 12 were given corn oil (SAL-veh). At week 3, all the rats underwent partial hepatectomies. At week 8, all rats were killed and tissues (liver and kidney) were collected. All the rats in the DEN groups consumed significantly less food and water at the end of first week than the rest of the animals. However, this difference levelled off at the end of the second week. A two-way analysis of variance of the effects of HCB treatment and DEN injections on body, liver and kidney weights was conducted among the different groups. Results of this analysis indicated that: (1) Only DEN treatment had a significant effect on body weight, (2) Only HCB treatment had an effect on kidney weight, (3) Both treatments significantly affected liver weights.

838 MORPHOMETRIC EVALUATION OF HEPATOCELLULAR RESPONSES TO PENTACHLOROBENZENE WITHIN THE FRAMEWORK OF ITO'S MEDIUM TERM LIVER FOCI BIOASSAY

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Ito's Medium Term Liver Foci Bioassay is a relatively short-term, reliable model for evaluating multistep carcinogenesis in the rat. The 8-week protocol utilizes diethylnitrosamine (DEN) given at week O as an initiator, followed by the repeated administration of test chemical(s) from weeks 2 to 8. At week 3, partial hepatectomies are performed in order to maximize cell proliferation. At week 8, the rats are sacrificed and evaluated for development of preneoplastic hepatocellular nodules. In order to determine the effect of the DEN administration and the partial hepatectomy on pentachlorobenzene

(PeCB) toxicity, we evaluated the histopathological and morphometric responses of the liver within the framework of Ito's Medium Term Liver Foci Bioassay. Treatment groups consisting of 8-week old male F344 Fischer rats were: DEN (200 mg/kg ip) + PeCB (0.4 mmol/kg); DEN + corn oil; saline + PeCB; and saline + corn oil. Another treatment group received DEN + PeCB, but did not undergo partial hepatectomy. The dosing of PeCB or corn oil was via gavage daily. At necropsy, liver weights were significantly increased among the DEN + PeCB group when compared to the control groups. In the DEN + PeCB group, relative liver weights were increased up to 80% when compared to control groups. Preliminary light microscopic examination indicated severe centrilobular hepatocellular hypertrophy. Numerous mitotic figures were present. Morphometric evaluation suggested that increased liver weight was due to both hypertrophy and hyperplasia of hepatocytes.

839 THE *IN OVO* MODEL OF HEPATOCARCINOGENESIS - COMPARISION OF TWO SPECIES

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The purpose of this study was the rapid induction of alterations that indicate a carcinogenic effect of a test chemical in the developing embryonic liver of bird embryos. Fertile turkey and quail eggs were injected with the hepatocarcinogenic nitrosamines nitrosodiethylamine and N-nitrosomorpholine prior to incubation. Embryos were removed from the shell four days before hatching and samples of the embryonic liver were processed for histologic investigation. Higher doses of the hepatocarcinogenic chemicals induced foci of altered hepatocytes that were similar to preneoplastic liver cell foci which have been observed in the liver of rodents after exposure to carcinogens. In addition hepatocytes with unusually large nuclei were observed after lower doses that were insufficient for the induction of phenotypically altered liver cell foci. Whereas the foci of phenotypically altered hepatocytes have been considered as direct precursors of hepatocellular tumors and reliable indicators of hepatocarcinogenicity, the significance of the observed nuclear enlargement is unclear. The *in ovo* model of hepatocarcinogenesis is suggested as a rapid and inexpensive experimental approach for the screening on carcinogenic effects and and contributes to the reduction of chronic cancer bioassays in rodents. Alterations induced in turkey eggs and quail eggs are presented and the advantages and disadvantages of turkey eggs versus quail eggs are discussed.

840 EFFECTS OF SIX PESTICIDES IN A LIVER MEDIUM-TERM BIOASSAY USING F344 MALE RATS

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Modifying effects of six pesticides (3 organophosphorus insecticides, 1 organophosphorus fungicide and 2 carbamate insecticides) were investigated using a medium-term liver bioassay based upon induction of preneoplastic glutathione S-transferase placental form positive hepatocytic foci in the rat liver. F344 male rats were initially injected with diethylnitrosamine (200mg/kg, single ip), and 2 weeks later were treated with EPN (75ppm and 150ppm), diazinon (500ppm and 1000ppm), phenthoate (500ppm and 1000ppm), iprobenfos (500ppm and 1000ppm), ethiofencarb (250ppm and 500ppm) or pirimicarb (200ppm and 400ppm) in the diet for 6 weeks and then killed, all rats being subjected to partial hepatectomy at week 3. A significant elevation of foci development was seen for 500ppm ethiofencarb. The results thus indicate that ethiofencarb at high dose, but not the other 5 pesticides, possesses promoting activity on rat liver carcinogenesis.

841 DOSE-DEPENDENT MODULATION OF CELL DEATH: APOPTOSIS VS NECROSIS IN THIOACETAMIDE HEPATOTOXICITY

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Apoptosis is programmed cell death, morphologically and biochemically distinct from necrosis. The objective of the present study was to examine thioacetamide-induced apoptosis over an early time course of 0 to 8 hr after administration of a 12-fold dose range (50, 150, 300 and 600 mg/kg, ip) of thioacetamide (TA). Male Sprague-Dawley rats (200–225 g) were used for the study. The incidence of apoptotic bodies (ABs) was determined by *in situ* end labelling, transmission electron micrography (TEM) and charge modification of Hsp 70. Light microscopic examination of liver sections revealed ABs as early as 2 hr. A dose-dependent increase in the incidence of ABs was seen with all doses till 4 hr. Thereafter, the incidence of ABs continued to increase in a temporal manner with 50 and 150 mg/kg, while it decreased in the rats treated with 300 and 600 mg/kg. Between 4–8 hr, while necrosis as assessed by serum ALT and histopathology declined in the 6- fold dose range (50, 150 and 300 mg TA/kg), it increased in a temporal manner with the 600 mg TA/kg. The findings for apoptosis were corroborated using TEM. Preliminary studies indicate an inverse relation between Hsp 70 expression and the incidence of apoptosis. Hsp 70 expression was significantly higher in the 600 mg TA/kg group compared to the lower doses. It was least in the group receiving 50 and 150 mg TA/kg where maximum apoptosis was noted. These findings collectively suggest that although the processes of apoptosis and necrosis are initiated simultaneously, the proportion of cells dying via either mechanism seem to be regulated by the dose of TA. Lower doses of TA seem to favor cell death via apoptosis, while higher doses favor cell death via necrosis. The molecular mechanisms underlying this "switch-over" are currently under investigation (Supported by ATSDR).

842 METHODS FOR FLOW CYTOMETRIC DETECTION OF APOPTOSIS AND ANEUPLOIDY IN PRIMARY AND IMMORTALIZED HUMAN AND MOUSE HEPATOCYTES

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Agents that injure liver cause cell death by necrosis and/or apoptosis, yet underlying mechanisms are poorly understood. Methods are needed to elucidate death-controlling events, particularly as they may relate to cell cycle. Human cells from transplanted or resected liver, HepG2 cells, and NIH Swiss mouse liver cells were examined by flow cytometry. Cell cycle stage and apoptosis were determined from DNA histograms of propidium iodide-stained nuclei. Nuclei were harvested by centrifugation (M-I) or trypsinization (M-II). M-I gave histograms of HepG2 nuclei that were debris-free in the apoptosis and hypodiploid aneuploidy region, with 7.4% apoptotic sub-G_0/G_1, 62.2% G_0/G_1, 9.3% S, and 22.3% G_2/M cells (coef.var.9%). Yet M-I histograms from cultured primary mouse and human cells had extensive sub-G_0/G_1, debris and a hyper-diploid G_0/G_1 shoulder of contaminating mitochondrial DNA. M-II resulted in 94.1% G_0/G_1, 2.4% S, and 3.5% G_2/M primary human cells (cv 3%) and 56.9% G_0/G_1, 10.5% S, and 32.7% G_2/M primary mouse cells (cv 3%). For these latter cells, M-II produced an unobscured apoptotic sub-G_0/G_1 region, and allowed detection of hypodiploid and hyperdiploid DNA of aneuploid cells (NIH ES 06345).

843 THE CALCIUM CHANNEL BLOCKER, DILTIAZEM, IS AN APOPTOTIC IMMUNOTOXIN

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We recently observed rapid induction of thymic apoptosis in male rats treated with diltiazem and three other calcium channel blockers. The objectives of this study were to determine whether diltiazem would also induce apoptotic effect in the thymus of another species and sex and in other lymphoid tissues. Species and gender specific thymic effects were assessed in adult female CA 1 mice at 3.5 hrs after treatment with diltiazem (90mg/kg,ip). Effects on non-thymus lymphoid tissues were assessed in adult male Sprague Dawley rats at 3.5 hrs after treatment with diltiazem (90mg/kg,ip). Sections of thymus, spleen, Peyer's patches and/or inguinal lymph nodes were stained with methylene blue or H & E for histopathological assessment by computer assisted image analysis. We found a three fold increase in the number of cells with morphological chracteristics of apoptotic cell death (i.e, cell shrinkage, chromatin condensation, and apoptotic bodies) in the thymus of diltiazem treated female mice. Thus the thymic apoptotic effect of this drug occurs in both sexes and multiple species. We found two to four fold increases in the apoptotic index in the spleen, Peyer's patches and lymph nodes of diltiazem treated male rats. This observation of apoptosis in lymphoid tissue of diltiazem treated rats suggests that this widely used drug could produce immunosuppressive effects by inducing apoptotic cell death of the peripheral lymphocytes. (Supported by NIEHS T 35-ES 07294)

844 AGE-DEPENDENT SENSITIZATION TO OXIDATIVE STRESS BY DIETARY FATTY ACIDS

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To test if an increase in dietary polyunsaturated fatty acids (PUFA) will increase liver cellular PUFA content and increase susceptibility to lipid peroxidation in an age-dependent manner, young (6 month) and old (24 month) male B6C3F1 mice were fed modified AIN-76 diets containing 5% corn oil (CO) or 19% menhaden oil plus 1% CO (FO) for two weeks. Mice fed FO were challenged with an acute systemic oxidative stress by ip injection of 125 mg Fe/kg bw as iron dextran. Mice (5 per group) were sacrificed 1, 5, and 24 hrs post-injection, livers removed, a portion fixed in formalin, and microsomal (MIC) and mitochondrial (MIT) fractions isolated. Serum iron increased significantly following Fe injections, peaking at 5 hrs in both age groups. Analysis for malondialdehyde and other thiobarbituric acid reactive substances (TBARS) in both MIC and MIT demonstrated significantly greater MIC TBARS in young mice, with the same temporal pattern as serum iron. Serum iron and MIC TBARS were significantly correlated, and slopes of plots by age group were significantly different by linear regression analysis. Histological examination for liver iron accumulation and apoptotic cell death showed peaks of both at 24 hrs, with a significantly greater percent apoptotic cells but no difference in iron accumulation in the young 24 hr group. Gas chromatography fatty acid analysis revealed that increased age and a FO diet independently increased membrane unsaturation, with MIT fatty acids from old mice significantly more unsaturated. Thus, livers of both age groups had similar iron exposure and iron accumulation, membranes of old mice were inherently more unsaturated, and yet there was significantly greater oxidative damage (TBARS) and cell death in the young livers. These findings suggest that the older animals have upregulated antioxidant defenses.

845 ACETAMINOPHEN-INDUCED HEPATOCELLULAR APOPTOSIS IN VIVO: ROLE OF Ca^{++}-ACTIVATED NITRIC OXIDE PATHWAY IN THE ABSENCE OF *bcl2* EXPRESSION

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Acetaminophen (APAP) is known to turn on apoptosis in addition to necrosis in liver *in vivo* (Yahya et al., 1995; Ray and Raje, 1995). Many studies indicate that production of nitric oxide (NO) and down regulation of *bcl2* may culminate apoptosis in some model systems. This study was designed to examine in liver whether: (i) toxic doses of APAP can generate NO, (ii) expression of *bcl2* is down regulated during hepatotoxicity, and (iii) apoptotic cell death is related to Ca++ accumulation and NO generation. In order to assess these events, male ICR mice (30–40g) were treated with hepatotoxic doses of APAP (600 mg/kg, ip) for 0, 4, 6, 12, 24 hr, and blood was collected upon sacrifice to determine ALT activity (hepatotoxicity). Portions of livers were processed separately for: (a) histopathological evaluation of apoptosis, (b) Nitrite accumulation (a stable end product of NO generation), (c) levels of citrulline (conversion product of L-arginine by NO-synthase), (d) *bcl2* expression, and (e) genomic DNA fragmentation. Results indicate that APAP treatment caused severe hepatotoxicity, a sharp rise in hepatocellular nuclear total Ca++ levels, massive accumulation of nitrite (48, 60, 100% at 4, 6, 24 hr respectively) and citrulline (32, 80, 189% at 4, 6, 24 hr, respectively) in the liver over time. Electrophoresis of hepatic DNA in the same time frame indicated ladder-like fragmentation consistent with histological findings of apoptotic cell death. A strong correlation was observed between nuclear total Ca++ levels with NO generation, or citrulline production. However, expression of *bcl2* was altogether absent in the liver proteins. All these *in vivo* events cumulatively indicate that APAP hepatotoxicity involves apoptotic cell death, with a possible role of Ca++-dependent generation of NO serving as an intracellular messenger.

846 FACILITATION OF APOPTOSIS BY AUTOLOGOUS SERUM AND RELATED IMMUNOSUPPRESSION IN THE SPLENOCYTE CULTURE

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Attempts are made to observe the apoptosis in splenocytes cultured under *in vitro* conditions in the presence of autologous serum. The addition of adult mouse serum (MS) to the culture of mouse splenocytes resulted in an accelerated decrease of viable cell number during initial 24 hours of culture as determined by trypan blue dye exclusion test and the propidium iodide staining method. Furthermore, the extent of DNA fragmentation, the hallmark of apoptosis, determined by agarose gel electrophoresis and the amount of fragmented DNA measured by ELISA method showed that the extent of apoptosis was clearly increased in splenocytes cultured in the presence of MS. Under the scanning and transmission electron microscopic observations, the large portion of splenocytes showed morphological characteristics of apoptotic cells such as apoptotic body, condensed chromatin and shrunken appearance. With the accelerated rate of apoptosis, the immunocompetence of splenocytes such as the antibody production, natural killer cell activity, and proliferation by mitogens was strongly suppressed. When analyzed by surface immunolabelling flow cytometry, the subsets of lymphocytes (B, T, CD4+ T and CD8+ T cells) were affected in a global nonselective manner. As determined by ultrafiltration, the molecular weights of apoptosis-facilitating factors present in MS appeared to be greater than 10 kDa. Upon fractionation with Sephadex G-200, the apoptotic factors were separated into 2 fractions.

In summary, results obtained in the present study indicate that some unidentified endogeneous macromolecules present in MS may produce the stimulatory effect on the apoptosis and cause immunosuppression of splenocytes under culture.

847 RAPID INDUCTION OF APOPTOSIS BY AFLATOXIN B1 IN RAT HEPATOCYTES *IN VITRO* AND *IN VIVO*

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Cell death produced by hepatotoxic doses of the genotoxic hepatocarcinogen aflatoxin B1 (AFB1) has been described as necrotic with occasional references to single cell necrosis. We found that in cultured rat hepatocytes AFB1 rapidly induces the chromatin margination, internucleosomal DNA cleavage, and formation of apoptotic bodies characteristic of programmed cell death. DNA fragmentation, as assessed by measurement of DNA in the neutral lysis fraction in alkaline elution assays and by gel electrophoresis, was observed as early as 3 hours after treatment with concentrations of AFB1 as low as 50 nM. Similar effects were observed after 3 hr treatment of hepatocyte cultures with 10 μM cycloheximide, a protein synthesis inhibitor previously shown to induce apoptosis in vivo in rat liver. However, an *in vitro* protein synthesis inhibition experiment indicated that the inhibitory effect of AFB1 on total protein synthesis is not likely to be responsible for apoptosis induction by AFB1. In vivo, single doses of 1–10 mg/kg of AFB1 in Sprague-Dawley rats produced dose- and time-dependent increases in the number of apoptotic cells and in the extent of necrosis in the liver as judged by serum alanine and aspartate aminotransferese activities and histologic examination. Increases in the apoptotic index were seen as early as 3 hours after treatment when there were no significant increases in serum enzyme activities. These results suggest that apoptosis induction is a significant feature of the acute hepatototoxic effects of AFB1 and may contribute to its tumorigenicity by stimulating regenerative cell proliferation and the resulting conversion of promutagenic DNA adducts to mutations in surviving cells.

848 DIFFERENTIAL INDUCTION OF APOPTOSIS IN B-LINEAGE LYMPHOCYTES COMPARED TO T-LINEAGE LYMPHOCYTES BY DIVERSE MOLECULAR LESIONS IN DNA

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B-lineage lymphocytes (BLL) are more sensitive than T-lineage lymphocytes (TLL) to cytotoxicity induced by drugs forming interstrand cross-links in DNA. We determined whether BLL are generally more sensitive than TLL to cytotoxicity induced by diverse molecular lesions in DNA, and whether this differential sensitivity involves triggering of apoptosis. Studies were performed with 5 different drugs in the chick embryo and in lymphoblastoid cell lines. Apoptosis was detected by Hoechst 33342 reporting of DNA condensation and segregation in cells. All drugs induced apoptosis in dose- and time-dependent manners. BLL were uniformly and substantially more sensitive than TLL to drug-induced apoptosis, reaching 75% of cells at 10 hrs of exposure. Drug-induced apoptosis in TLL did not exceed 30% of cells. Different potency for inducing apoptosis was observed with topotecan (TPT) and mitomycin C (MMC) the most effective, and MMS the least. The potency ranking for three of the drugs was the same *in vivo* and *in vitro* - TPT>MMC-

>MMS. These results demonstrate the multidrug sensitivity of BLL compared to TLL, and this involves the differential triggering of apoptosis.

849 CHLOROETHYL ETHYL SULFIDE (CEES) CAUSES BOTH NECROSIS AND APOPTOSIS IN DIFFERENTIATED PC12 CELLS

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Sulfur mustard-induced vesication involves necrotic cell death. Recently apoptopic cell death has been studied as a possible mechanism associated with necrosis. Rat pheochromocytoma (PC12) cells are a well characterized cell model known to undergo apoptosis in response to certain toxicants. To evaluate the role of apoptosis in mustard-induced cytotoxicity, differentiated PC12 cells were exposed in increasing concentrations (10^{-7}M-10^{-2} M) of chlorolethyl ethyl sulfide (CEES) for 24, 48, and 72 hrs. Based on cell morphology and LDH release, no evidence of cytotoxicity was observed at 24 hrs. At 48 and 72 hrs CEES produced significant morphological changes and increased LDH release, thereby showing a delayed toxic effect. Both morphological and cytotoxic effects occurred in the concentration range of 10^{-5} to 10^{-4}M. DNA fragmentation, TUNEL and transmission electron microscopy (TEM) were used to confirm apoptosis upon CEES treatment. At 48 hrs. 10^{-4} M of CEES oligonuclide laddering was noted on electrophoresis confirming DNA fragmentation. TUNEL produced dark brown staining of cellular and nuclear membranes and the chromatin of the cells. Based on these studies the relative degree of apoptosis vs necrosis was 5×10^{-4} M at 48 hrs. and 10^{-5} M at 72 hrs. The incidence of apoptosis decreased with increasing CEES concentrations suggesting necrosis occurs at higher concentrations. It is concluded that CEES causes both necrotic and apoptotic cell death in the differentiated PC12 cells and is a useful model to study CEES-induced apoptosis. (Supported by U.S. Army Medical Res. Devel. Command Contract DAMD 17-93-C-3038)

850 THE ANTI-PROLIFERATIVE EFFECTS OF SELENOMETHIONINE AGAINST HUMAN TUMOR CELLS

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The efficacy of dietary selenium supplementation is currently being evaluated in intervention trials at The Arizona Cancer Center. However, the biological mechanisms underlying the cancer chemopreventative effects of selenium supplementation have yet to be elucidated. Selenium metabolism and polyamine biosynthesis are linked in their common requirement for S-adenosylmethionine. The effects of selenomethionine on the growth and polyamine biosynthesis were examined in the human lung cancer cell line A549 and the human colon cancer cell line HT-29. We found, using a sulforhodamine B (SRB) assay, that selenomethionine can inhibit tumor growth (both in lung and colon tumor cells) in a dose-dependent manner. Furthermore, we found that tumor cells were more sensitive to these effects than normal diploid fibroblasts. At 24 hours, polyamine content of A549 and HT29 cell lines was decreased at a dose of selenomethionine that inhibited growth at 50% for each of the cell lines. Finally, we detected apoptotic cells at 24 hours after treatment with selenomethionine (IC_{50} dose of 65 μM for A549's and 130 μM for HT-29's) using the TUNEL method and morphological assessment of hematoxylin and eosin (H&E) stained cytospin slides. These data suggest that selenomethionine administration might exert its anti-carcinogenic effects via polyamine depletion which leads to apoptosis in human tumor cells. Supported in part by SWEHC: NIEHS Center Grant P30-ES-06694 and NCI CA49764

851 CHEMICAL-INDUCED GENE EXPRESSION: STUDY OF METHYLMERCURY IN DIFFERENTIATING NEURONAL CELLS

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One of the mechanisms for cells to cope with physical or chemical insult is by inducing genes involved in protecting or repairing cellular damages. In these studies we evaluated the effects of MeHg on gene expression in neuronal differentiating cells. Micromass primary neuronal cells (CNS) was used for the study. Genes investigated include DNA damage-responsive genes (Gadd genes) and an oxidative stress-inducible gene, gamma-glutamylcysteine synthetase (Gamma-GCS). MeHg exposure (2 uM) for 24 hour was used in *in vitro* experiments. This dose caused a 4 fold decrease in the number of cells passing through the cell cycle. mRNA was isolated for control and MeHg exposed cells. MeHg exposure caused a 5–10 fold increase in Gadd 45 and 3–5 fold increase in Gadd 153 expression in CNS cells. For *in vivo* comparison, brain tissue was isolated from adult mice chronically exposed to 10 ppm MeHg over 4 weeks. This dose of MeHg caused developmental toxicity *in vivo*. Results showed a similarity in MeHg induced expression of Gadd 45 between brain tissues *in vivo* and differentiating neuronal cells *in vitro*. Constitutive expression of these genes during differentiation was also examined and Gadd 45 showed decreases with neuronal differentiation. These observations may indicate a possible role of these genes in regulating cell cycle during differentiation. Small increases in GCS expression were seen *in vivo* with MeHg exposure and *in vitro* in CNS cells. This may reflect changes in oxidative states of these cells.

852 APOPTOTIC CELL DEATH IN RETINAS OF DEVELOPMENTALLY LEAD-EXPOSED RATS RESULTS IN THE PRODUCTION OF LARGE 50–700 kbp, BUT NOT INTERNUCLEOSOMAL, DNA FRAGMENTS

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Ultrastructural studies show that lead exposure during development (days 0–21) results in the daily loss of rods and bipolars such that by 45 days of age 22% and 30% of these retinal cells died by apoptosis, respectively. To determine the biochemical mechanisms, we developed methods to examine the temporal pattern and extent of genomic DNA fragmentation during and following lead exposure. Neural retinas from 2–21 and 45 day old control and lead-exposed rats were dissociated into cellular suspensions and agarose plugs were prepared. The occurrence of large (50, 300 & 700 kbp), and internucleosomal (ladders) DNA fragments was examined using pulse-field and standard gel electrophoresis, respectively. Overall, DNA was predominantly genomic with age- and lead-related changes in the relative amounts of large kbp fragments. In developing lead-exposed rats the pattern of large fragmentation was similar to controls, however, the amount of all large fragments was increased at 2–21 days of age. At 45 days, there were no large fragments in controls whereas lead-exposed retinas had 300 kbp fragments. Ladders were not seen in control or lead-exposed retinas: the latter supported by our finding of a Pb^{2+}-induced inhibition of Ca^{2+}-Mg^{2+} endonuclease. These results, combined with our ultrastructural data, demonstrate that lead-induced apoptosis temporally correlates with the occurrence of large kbp fragmentation. Thus, fragmentation of genomic DNA into large kbp fragments (chromatin loops), but not into ladders, appears to be an early, essential and irreversible step in the apoptotic process. Supported by NIH Grant ESO3183.

853 MERCURY (II) CHLORIDE INDUCED APOPTOSIS IN THE CULTURED PORCINE RENAL CELL LINE LLC-PK_1

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Mercury chloride, a well characterized renal toxin, was used to induce apoptosis in a cultured renal proximal tubule cell line, LLC-PK_1. Apoptosis in response to 35μM $HgCl_2$ was confirmed by observation of morphological features characteristic of apoptosing cells as well as fragmentation of chromosomal DNA into 200 base pair fragments. Ten percent of LLC-PK_1 cells in a monolayer underwent apoptosis. These cells detached from the culture flask during apoptosis. Measurement of transepithelial resistance (TER) was used as a functional assay of junctional complex integrity in a novel approach to characterize pre-apoptotic events in this cell line. Monolayers of LLC-PK_1 cells that contained apoptosing cells experienced a transient decrease in TER followed by a recovery of TER to the initial levels. The decrease in TER was accompanied by a decrease in transepithelial transport as assessed by loss of hemicysts within the monolayer. This data indicates a temporary loss of junctional complexes within the monolayer during apoptosis. This work was supported by the Superfund Basic Research Program Project Grant NIEHS #ES4940, project #12. The support of the Southwestern Environmental Health Sciences Center (NIEHS Center Grant P30ES-06694) was also instrumental in completion of this work.

854 INDUCTION OF APOPTOSIS BY BENZENE METABOLITES IN HL60 AND CD34+ HUMAN BONE MARROW PROGENITOR CELLS

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A high myeloperoxidase (MPO)/NAD(P)H: quinone oxidoreductase (NQO1) ratio represents a shift toward bio-activation which may be important in determining cell susceptibility to the toxicity of phenolic metabolites of benzene. Two cell types with high MPO/NQO1 ratios were used as model systems to explore a possible role for apoptosis in the myelotoxicity of these metabolites. HL60 cells, a human promyelocytic leukemia cell line, were treated with either phenol, catechol, or hydroquinone (HQ) and then stained with Hoechst 33342 and propidium iodide and subjected to fluorescent microscopy. Cells with nuclear condensation and fragmentation were scored as apoptotic and VP-16 (40 μM) was used as a positive control. Both catechol and HQ induced marked time- (0-24h) and concentration-(25-100 μM) dependent apoptosis, while phenol (750 μM) did not. Under these conditions, no significant necrosis was observed. The induction of apoptosis was confirmed by internucleosomal cleavage of DNA, assessed by agarose gel electrophoresis. CD34+ cells treated with 100 μM HQ for 18h were stained and subjected to fluorescent microscopy as above. The percentage of cells exhibiting nuclear condensation and/or fragmentation as well as high intensity staining significantly increased. These data show that apoptosis can be induced in both HL60 and CD34+ human bone marrow progenitor cells by benzene metabolites and suggests inappropriate apoptosis may contribute to benzene myelotoxicity. (Supported by ES 04112 and The American Petroleum Institute).

855 QUANTITATION OF APOPTOSIS IN MOUSE LIVER

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Recent data have indicated that cell death regulation is as essential as cell proliferation in multistage carcinogenesis. We quantitated apoptosis in hematoxylin and eosin (H&E) stained liver sections derived from control B6C3F1 female mice and mice administered the carcinogenic agent furan for three weeks (8 and 15 mg/kg, 5 d/wk). Detection of apoptosis by H&E was used to obtain apoptotic indices as well as to study different stages of apoptosis. Issues relating to the detection and quantitation of hepatocyte apoptosis were addressed. The apoptotic index was approximately 0.1% in control animals. Furan was found to cause a dose-related increase in apoptosis (~1.7% in high-dose mice). Both treated and nontreated mice exhibited a substantial interindividual variability in liver apoptosis that coincided with the mitotic index. The incidence of early apoptotic hepatocytes (i.e. cells displaying condensed chromatin marginated to the nuclear membrane) was very low (<0.01%). Approximately 90% of all detectable apoptotic bodies were located intracellularly. About one-half of all apoptotic bodies were found to have visible condensed chromatin, and approximately 2 apoptotic bodies were estimated to correspond to one apoptotic cell. These results describe and quantitate the level of hepatocyte apoptosis in control female mice and demonstrate the induction of apoptosis in furan-treated mice. These data form a basis for the comparison between H&E and other slide-based methods for apoptosis detection and quantification.

856 CADMIUM-INDUCED APOPTOSIS IN MOUSE LIVER

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Apoptosis is a process of active cell death, distinct from necrosis and characterized by specific morphological and biochemical features. It is induced by certain physiological and pathological states. Although the acute hepatotoxic effects of cadmium (Cd) are well described, little is known about its ability to induce apoptosis in the liver. With this in view, mice were injected *i.p.* with 3.4 mg/kg of Cd. Their livers were removed 1.5–48 hrs later and examined by light microscopy. Cd induced a time-dependent rise in Apoptotic Index, which peaked at 9–14 hrs and then fell. The time course of apoptotic cell death index, monitored by quantitation of the oligonucleosomal DNA fragments, mirrored the pattern obtained by histopathology and a commercial *in situ* apoptotic DNA detection kit. Liver necrosis, as shown by histology and serum alanine aminotransferase assay, was evident 9–48 hrs after Cd injection. Necrosis was sustained whereas apoptosis was transient. Replacement of liver tissue by blood lakes (peliosis hepatis) was observed after 14 hrs. The Mitotic Index showed a very gradual rise over time, and became significant at 48 hrs, indicating liver cell regeneration at this time. These data demonstrate that apoptosis is a feature of Cd hepatotoxicity and is resolved by 24 hrs while necrosis persists longer. (Supported by NIH Grant ES-01142)

857 CADMIUM AND CHROMIUM INDUCED OXIDATIVE STRESS AND PROGRAMMED CELL DEATH IN CULTURED J774A.1 MACROPHAGE CELLS

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Sodium dichromate [Cr VI] and cadmium chloride [Cd II] are known to induce cytotoxicity and mutagenesis. This *in vitro* study was designed to focus upon toxic and apoptopic potential of these cations on cultured J774A.1 cells. The cells were incubated with 0.20, 0.40 and 0.60 μM concentrations of these cations for 0, 24 or 48 hrs at 37°C. Cr VI- or Cd II-induced changes in cell morphology were detected using phase contrast microscopy. The overall intracellular oxidized states of cells were measured at an excitation wavelength of 513 nm by Laser Scanning Confocal Microscopy using 2, 7-dichlorofluorescein diacetate (DCFD) as the probe. Concentration dependent increases in fluorescence intensity were observed. Signal was quantitated by integrating fluorescence over a user defined area cell number. Approximately 3.6–8.2 fold increases in fluorescence intensity were observed following treatment with Cr VI or Cd II ions. Concentration-and time-dependent effects of Cr VI and Cd II ions on succinate dehydrogenase, a marker of mitochondrial electron transport chain, were determined. Significant increases in enzyme activity were observed with both cations at 0.40 and 0.60 μM concentrations at 24 hrs, while a significant decrease in enzyme activity was observed at 48 hrs. Cr VI produced a more pronounced effect as compared to Cd II ions. Fragmentation of nuclear DNA is a biochemical hallmark of programmed cell death (apoptosis), which was assessed using the TUNNEL (TdT-mediated dUTP-biotin nick end labelling) method employing fluorescent microscopy. Concentration-and time-dependent induction of apoptosis were observed with both cations. Cr VI was shown to induce toxic effects at lower concentrations than Cd II ions. These results clearly indicate that both cations are toxic, producing oxidative tissue damage and apoptosis.

858 SPECIES COMPARISON OF *IN VITRO* SKIN PENETRATION OF BENZOYL PEROXIDE (BPO)

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The *in vitro* percutaneous penetration of BPO suspended in an aqueous medium was compared between skin samples obtained from F344 rat, B6C3F1 and SKH1(hr/hr) hairless mice, and human cadaver skin in an infinite dose, flow through diffusion apparatus. The objective of these studies was to determine the difference in penetration of BPO between rodent and human skin. Concentrations of BPO ranging from 2.5 to 50% w/v were applied to skin discs (1.77 cm^2) and, based on time to achieve a steady-state rate of penetration, effluent samples were collected at 30 min intervals over 6 hr. The effluent (minimum essential medium), with and without polyethylene glycol, was analyzed for BPO and its primary metabolite, benzoic acid (BA) using liquid chromatography with a UV detector. Since no BPO was detected in the effluent in the presence or absence of polyethylene glycol below the HPLC limit of detection (i.e., <50 ng/ml), the concentration of BA was used to calculate indices of percutaneous penetration of benzoyl peroxide. There was no difference in the cumulative amount (μ/cm^2) of BPO delivered from 2.5 to 50% w/w gels within a given species. However, the percutaneous penetration of BPO through skin samples obtained from the SKH1 hairless mice was significantly ($p<0.001$) greater than human, F344 rat or B6C3F1 mouse. Penetration of BPO through F344 rat and B6C3F1 mouse skin was equivalent and slightly but not statistically greater than human cadaver skin in these studies. The solubility of BPO in the aqueous vehicle may be the rate limiting step in percutaneous penetration since comparable rates of penetration were obtained from suspensions containing 2.5 and 50% BPO. Most important, the penetration of BPO is 7–10 times greater through the SKH1 (hr/hr) hairless mouse than through F344 rat, B6C3F1 mouse and human cadaver skin. Thus, applied dose of BPO to the skin of the hairless mouse delivers a greater amount of biologically active material to the basal cell layers and beyond as compared to other rodents and human skin. This skin penetration difference should be considered when results from topical studies with SKH1 (hr/hr) hairless mice are extrapolated to human exposure.

859 ENHANCED ACTIVATION STATE OF RAS IN PSORIATIC LESIONS

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Ras plays an important role in normal cellular proliferation, differentiation and maligant transformation. Ras is a proximal component of a signalling cascade linking tyrosine kinase growth factor receptors to alterations in gene expression. Ras is converted from an inactive GDP-bound state to an active GTP-bound state as a direct in response to growth factor receptor activation. Psoriasis is a common skin disease characterized by epidermal hyperproliferation and inflammation. Utilizing a recently described method (P.N.A.S. 1995, 92: 1097), we have determined relative levels of inactive and active Ras in normal and psoriatic epidermis. Ras proteins in human keratome biopsies were immunoprecipitated, and Ras-bound GTP and GDP were quantified. GDP was converted to [^{32}P]GTP, utilizing [^{32}P]ATP and NDP kinase, and [^{32}P]GTP was quantified by phosphoimager. GTP was converted to ATP using GDP and NDP kinase, and ATP levels were quantified by luciferase-catalyzed light emission. Normal human epidermis contained 59 ± 15 and 975 ± 211 fmol (n = 7) of Ras-bound GTP and GDP per mg protein, respectively. Thus, 6% of Ras was in the active state. In contrast, psoriatic epidermis contained 173 ± 37 and 946 ± 79 fmol (n = 4) of Ras-bound GTP and GDP per mg protein, respectively, yielding 15.4% of Ras in the active state. These data provide direct evidence for Ras activation in a human hyperproliferative disease and are consistent with previously reported elevations in growth factor levels in psoriatic lesions.

860 A RAT MODEL FOR ASSESSING THE TOXICITY OF DERMALLY APPLIED COMPOUNDS ADMINISTERED CONTINUOUSLY FROM NEONATE TO SEXUAL MATURITY

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Animals and humans may be exposed dermally to chemicals soon after birth. Few studies have been reported in which rats have been exposed dermally to chemicals starting as neonates and continuing through to sexual maturity. In the studies reported here, various formulations were applied daily at a dose of 2 g/kg, to the dorsal skin of 4-day old rats and continued for approximately 90 consecutive days. The pups were housed with their dams until weaning after which they were housed individually. The animals were observed daily and body weights measured twice weekly until weaning, then once a week thereafter. Hematologic and clinical chemistry parameters as well as weights and histology of selected organs were evaluated when the animals were approximately one and three months of age. No evidence of systemic toxicity developed during the course of the study. However, time-dependent dermal irritation occurred in the animals exposed to the test formulations. These studies demonstrate that this rat neonate model may be used to determine the potential toxicity of chemicals or formulations when chronic exposure begins soon after birth. Also the control group data demonstrates the changes that occur in the various parameters evaluated in rats at one and three months of age.

861 PERCUTANEOUS ABSORPTION AND SKIN DISPOSITION OF [^{14}C-UL] PENTACHLOROPHENOL (PCP) IN SWINE: DOSING VEHICLE AND OCCLUSION EFFECTS

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The effects of dosing vehicle and occlusion on the percutaneous absorption and cutaneous disposition of [^{14}C] were investigated following topical occluded or non-occluded dose of [^{14}C]PCP onto abdominal skin (300μg/10μgCi; 40μg/cm^2) in weanling pigs using different dosing vehicles including ethanol, acetone, water, and soil. The blood and total (urine + feces) excretion profiles were also examined after PCP IV injection. After dosing PCP in ethanol onto the non-occluded abdomen skin (n = 3), the average relative absorption [excretion$_{topical}$]/[excretion$_{iv}$] *100%) by 17 days was 53.5% with a total recovery of 70.2 ± 13.9%. After IV dosing, a half-life in plasma and blood was determined at 96–120 hr, 109.1 ± 20.6% of the applied dose was recovered and 16.0 ± 4.69% of the initial dose was excreted almost equally via urine and feces. Time of maximal excretion rate in urine after IV dosing was determined at 61.3 ± 26.6 hr. After IV dosing, the highest concentration was found in the liver at 133.0 ± 32.9 ng/g, followed by the kidney at 27.3 ± 6.6 and the ovary at 26.3 ± 0.6 ng/g. A similar tissue distribution pattern was also found after topical application, indicating the potential for female reproductive system toxicity. The same parameters were also determined after several other dosing vehicles. The dosing vehicle and occlusion are factors which must be considered when assessing the risk of exposure to topically applied compounds. Supported by USEPA grant CR 824077-01-0.

862 SKIN ABSORPTION METHODOLOGY: IMPROVING THE LIPOPHILICITY OF THE RECEPTOR FLUID AND THE ACCURACY OF THE SKIN VIABILITY ASSAY

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The receptor fluid used in an *in vitro* percutaneous absorption study should not only serve as a receptor for the penetrant but also preserve the viability of the skin. Lipophilicity of the receptor fluid plays a crucial role in facilitating the release of a lipophilic compound from skin into the receptor fluid. Until now, 4% bovine serum albumin (BSA) has routinely been added to HEPES-buffered Hanks' balanced salt solution (HHBSS) to increase the aqueous solubility of lipoidal compounds. Hydroxypropyl-β-cyclodextrin (HPCD) was compared with BSA for its ability to solubilize lipid material and also maintain skin viability. Concurrent with the development of a more lipophilic receptor fluid, a second skin viability assay was evaluated as an alternative test to the lactate assay. Ultimately, the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was shown to be a more accurate and reliable method for assessing skin viability than the lactate assay. HHBSS + 2% HPCD proved to be more suitable than HHBSS + 4% BSA. With HHBSS + 2% HPCD, better agreement was found between the results of *in vitro* and *in vivo* skin absorption studies of a fragrance ingredient, and skin viability was maintained for the entire 24-hour study.

863 *IN VITRO* DERMAL ABSORPTION OF COUMARIN IN RAT AND HUMAN SKIN

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Coumarin is widely used as a fragrance in cosmetics, perfumes and soaps. Concerns about its safety have been raised by toxicity testing conducted by the National Toxicology Program. Therefore, we initiated studies to measure the extent of coumarin absorption and metabolism in skin. [^{14}C]Coumarin (approx. 0.5 μCi/cell) absorption in skin was measured by using two vehicles, ethanol (15 μL/cm^2) and an oil-in-water emulsion (2 mg/cm^2). Absorption was determined for 24 hr by using flow-through diffusion cells (0.64 cm^2) with a receptor fluid consisting of HEPES-buffered Hank's balanced salt solution (pH 7.4). Coumarin metabolism was determined by HPLC in rat skin experiments. In rat skin (n = 3), the percentages of applied dose absorbed were 54.9 ± 0.63 (mean ± SEM) and 86.8 ± 5.4 for the ethanol and emulsion vehicles, respectively, with approximately 5% remaining in skin. In human skin (n = 2), the percentages of applied dose absorbed were 64.4 ± 0.29 and 98.0 ± 5.3 for the ethanol and emulsion vehicles, respectively, with approximately 1% remaining in skin. The extent of skin absorption was greater from the emulsion vehicle than from the ethanol vehicle in both human and rat skin. Coumarin rapidly penetrated both rat and human skin with > 75 and > 95%, respectively, of the absorbed dose found in the receptor fluid within 6 hr. No evidence of coumarin metabolism was found in rat skin or receptor fluid fractions. These studies indicate that coumarin absorption is significant in skin. Systemic coumarin absorption must be expected after dermal contact with coumarin-containing products.

864 COMPARISON OF *IN VITRO* AND *IN VIVO* HUMAN SKIN RESPONSES TO CONSUMER PRODUCTS AND INGREDIENTS WITH A RANGE OF IRRITANCY POTENTIAL

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Human skin equivalent cultures are being investigated as a pre-clinical skin irritation screen to aid in the design of safe and efficient human studies. Our approach has been to directly compare *in vitro* to *in vivo* human skin responses using historic or concurrent skin response data for products and ingredients including surfactants, cosmetics (i.e., liquid make-up), antiperspirants and deodorants (AP/DO). The *in vivo* data consisted of visual scores (i.e., erythema

and edema) from skin patch tests and diary accounts of skin irritation from product use studies. For the *in vitro* studies we evaluated cornified, air-interfaced human skin cultures (Epiderm™) using methods to parallel human clinical protocols by topical dosing of neat or diluted test substances to the stratum corneum surface of the skin cultures. In the *in vitro* studies we evaluated endpoints that we have shown previously to be relevant to human skin irritation *in vivo*, including the MTT metabolism assay of cell viability, enzyme release (lactate dehydrogenase and aspartate aminotransferase) and inflammatory cytokine expression (Interleukin-1α). For surfactants, dose-response curves of MTT cell viability data clearly distinguished strongly irritating from milder surfactants, and rank ordered irritancy potential in a manner similar to *in vivo* 3-patch test results. For AP/DO products, all the *in vitro* endpoints correlated well with consumer reported irritation ($r = 0.78-0.94$), with IL-1α release showing the greatest capacity to distinguish irritancy over a broad range. IL-1α also showed the best prediction of human skin scores from 14-day cumulative irritancy tests of cosmetic products. These results confirm the potential value of cornified human skin cultures as an *in vitro* pre-clinical screen for prediction of human skin irritation responses, and identify the need to customize *in vitro* endpoints for prediction of different product classes.

865 USE OF THE CYTOSENSOR MICROPHYSIOMETER TO PREDICT RESULTS OF A 21-DAY CUMULATIVE IRRITATION PATCH TEST IN HUMANS

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The human 21-Day Cumulative Irritation Patch Test (21-CIPT) (Lanman, et al., 1968) is a standard test of new products intended for repeated dermal contact. However, duration and cost limit its use to screen multiple formulas early in the product development cycle. Thus, there is need for a rapid, relatively inexpensive test that predicts performance on the 21-CIPT. The Cytosensor Microphysiometer (μϕ) (Molecular Devices Corp., Menlo Park, CA) was investigated for this application. It indirectly measures metabolic rate changes in L929 cells as a function of test article dose, by measuring the acidification rate in a low buffer medium. The cells are exposed to increasing concentrations of test material in a cycle of exposure (13.5 minutes), wash (6 minutes), and measurement (0.5 minutes). The dose producing a 50% reduction in metabolic rate (MRD_{50}), relative to the baseline, is used as a measure of toxicity. Nineteen formulas were tested via the μϕ and 21-CIPT. One was a product with over five years successful market experience. Comparing the results from both tests, samples with an MRD_{50} greater than 50mg/ml provided a 21-CIPT score consistent with a product that performs satisfactorily in the market. When the MRD_{50} was greater than 80mg/ml, the 21-CIPT score was usually zero. The μϕ may be more sensitive than the 21-CIPT for ranking minimally irritating materials. The μϕ is useful as a screen for predicting the performance of a formula on the 21-CIPT.

866 APPLICATION OF HUMAN SKIN/NUDE MOUSE MODEL FOR PERCUTANEOUS ABSORPTION STUDIES OF OCTAMETHYLCYCLOTETRASILOXANE (D4)

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D4 is a widely used ingredient of cosmetics (antiperspirants, skin and hair care products). The objective of the present study was to evaluate dermal absorption and skin distribution of the octamethylcyclotetrasiloxane using human skin transplanted onto nude mice. Single doses of pure ^{14}C-D4 (14.7 mg/cm^2) were applied to grafts for 24 hours, using a modified Susten skin depot, and animals were monitored for the next 2 days. ^{14}C-D4 was measured in human and animal skin, carcass and excreta. Most of the applied substance evaporated from the site of exposure (80.1%). The minimal total absorption of D4 was 8.0% of recovered dose. The application site contained 0.5% of the dose. Low levels of D4 were detected in feces (4.8%), urine (0.5%), CO_2 traps (1.1%) and carcass (0.9%). D4 concentrations in human epidermis, dermis and adipose tissue were 76.0, 6.1 and 0.8 μg/g, respectively. Our studies demonstrated a low degree of D4 absorption through the skin using the human skin/nude mouse model and only slight retention of the agent in human skin.

867 EVALUATION OF A HUMAN SKIN EQUIVALENT MODEL AS A POTENTIAL *IN VITRO* SYSTEM TO EVALUATE THE CONTACT SENSITIZATION POTENTIAL OF CHEMICALS

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Human skin equivalent cultures are currently being investigated as *in vitro* models to predict the contact sensitization potential of chemicals on human skin. One such culture is the ATS SKIN²™ Model ZK1300 consisting of dermal fibroblasts and differentiated epidermal keratinocytes from neonatal foreskin. A functional stratum corneum is present and allows simulation of human topical exposures as well as application of non-aqueous materials. Our objective is to assess the ability of irritants and contact allergens to modulate cytokine message in the SKIN²™ cultures. Levels of cytokine gene expression were determined using reverse transcriptase polymerase chain reaction (RT-PCR) and liquid hybridization for semi-quantitation.

Application of the contact allergens DNCB and oxazolone (OXA), and the irritants nonanoic acid (NA) and benzalkonium chloride (BC) increased the steady state message levels of the cytokines IL-6, IL-8 and GM-CSF greater than 2 fold over vehicle control. Both NA and BC increased the steady state levels of TNF-α, while DNCB decreased the TNF-α steady state levels. The steady state level of message for IL-15 was increased by NA only. No expression in IL-12 p40 was detected with chemical treatment. These changes vary for each chemical and are dependent on time of exposure and dose. These preliminary experiments demonstrate that allergens and irritants can modulate cytokine message levels in a time- and concentration-dependent manner in the SKIN²™ cultures. However, no clear differences between sensitizers and irritants were evident from these studies.

Further information will be required to determine if this *in vitro* culture system is able to identify the sensitization potential of materials and differentiate them from irritants.

868 SKIN ABSORPTION OF SYNTHETIC MUSK FRAGRANCE CHEMICALS

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Sponsor: R Ford

The synthetic musks, musk ketone (MK), musk xylene (MX), 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran (HHCB) & 6-acetyl-1,1,2,4,4,7-hexamethyltetraline (AHTN) find widespread use as fragrance chemicals and so routinely come into contact with human skin. For risk assessment purposes, it is of importance to determine the extent to which these compounds are absorbed across the skin into the systemic circulation. We have investigated the comparative absorption of the four radiolabelled musk chemicals (applied in a typical fragrance vehicle of 75% v/v ethanol: 25% v/v diethylphthalate) in fresh, full-thickness, male F344 rat skin using an *in vitro* system (Hotchkiss *et al.*, Fd Chem Toxic, 28, 443-447, 1990). All four musks were poorly absorbed through unoccluded rat skin into the receptor fluid (50% v/v aqueous ethanol). Absorption of the structurally related nitroaromatic musks, MK and MX, was $3.28 \pm 2.35\%$ (mean ± SD, $n = 4$) and $0.61 \pm 0.16\%$ ($n = 8$) at 24h, respectively, whilst absorption of the chemically related polycyclic musks, HHCB and AHTN, was $0.07 \pm 0.03\%$ ($n = 4$) and $0.28 \pm 0.14\%$ ($n = 3$), respectively. Significant amounts of radioactivity (approx. 50-60% dose) were recovered from within the skin at the end of the experiment. These data indicate that the musks are well absorbed into the skin (due to their high lipophilicity: log $P_{o/w}$ MK 3.78, MX 3.71, HHCB 6.06, AHTN 6.25), although systemic absorption of the compounds over 24h is likely to be low.

869 STRATUM CORNEUM PERMEABILITY COEFFICIENT DETERMINATION FOR VOLATILE CHEMICALS USING THERMAL GRAVIMETRIC ANALYSIS METHODS

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The Stratum Corneum (SC) can be the primary barrier to transdermal penetration of volatile organic occupational and environmental chemicals. Much of the quantitative information to describe or compare the movement of chemicals through the SC is contained in the SC permeability coefficient. The SC permeability coefficient has the units of cm/hr and is a composite of the diffusion coefficient, SC to air partition coefficient and the diffusion pathlength distance. Thermal gravimetric analysis (TGA) of SC during exposure to volatile organic chemicals was used to estimate SC permeability coefficients.

Whole thickness human or rat skin was excised and dermatomed to a depth of 0.025 in. The skin was treated with trypsin for 2 hours at 37°C in order to separate the SC from the underlying viable epidermis. The SC was dried and stored in a dessicator. Five milligrams of SC were placed into the titanium sample pan of the TGA cell. The cell was maintained at 32°C and purged with 50 mL/min of air and the weight was monitored until equilibrium was reached. The flow was then stepped to 1.225 L/min and maintained until equilibrium was again reached. Then 10000 ppm of chemical vapor was introduced into the TGA cell at 1.225 L/min. The weight increase per unit time was evaluated using a non steady-state diffusion equation in order to obtain a SC diffusion coefficient and the equilibrium phase was used to obtain a SC to air partition coefficient. The information derived from the TGA combined with SC thickness measurements from microscopic analysis allowed for estimation of SC permeability coefficients. This approach was proven useful for determining volatile organic chemical SC permeability coefficients in different species and under different temperature and exposure conditions.

870 IMMUNOHISTOCHEMICAL LOCALIZATION OF TUMOR NECROSIS FACTOR ALPHA IN PORCINE SKIN FOLLOWING LIDOCAINE IONTOPHORESIS

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Iontophoresis involves the delivery of a charged compound across the skin using an electrical current. Previously, we have shown that skin under the active electrode exhibits erythema, edema, and petechiae as well as an epidermal alteration in response to lidocaine iontophoresis. Tumor necrosis factor alpha (TNF-α) expression was quantitated by immunohistochemistry (IH) in 4 weanling Yorkshire pigs. Two pigs were iontophoresed with 4% lidocaine hydrochloride at 0.9 or 2.7 mA for 10 or 30 minutes and sampled immediately or 4 hours post-exposure. In addition, lidocaine controls exposed for 30 minutes and normal pig controls were harvested. TNF-α staining showed no differences between iontophoretic or control sites. Two additional pigs were tested in order to better isolate treatment effects from possible systemic effects (sampling and/or iontophoretic) or pig variability. IH showed a slight increase in lidocaine controls over normal pig skin. When the current was applied to the pig, the active sites as well as the normal control sites showed a marginal increase in TNF-α expression. In conclusion, iontophoresis does not significantly increase the expression of TNF-α.

871 MATHEMATICAL MODELING OF SKIN DIFFUSION CELLS.

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Determining the rate of penetration of chemicals through skin is necessary to assess the hazard with dermal exposure to chemicals in the occupational or environmental setting. Small glass diffusion cells with donor and receptor sides separated by excised skin are commonly used to determine the rate of penetration of chemicals through skin. Flux (mg cm^{-2} hr^{-1}) or permeability coefficient (cm hr^{-1}) are common ways to express the amount of chemical which comes through the skin at steady state. One problem with these studies is that steady rates of absorption must be reached in order to accurately calculate flux from the slope of total absorption versus time plot. The driving forces for penetration, according to Fick's Law: Flux $= \frac{K_m D}{\delta}(\Delta C)$ are constants, where K_m is the membrane partition coefficient, D is diffusivity and δ is membrane thickness, and ΔC is the difference in concentration across the membrane. It is normally necessary to put excess chemical in contact with the skin and wait sufficient time to approximate steady state in order to make flux measurements. The purpose of this investigation was to determine if estimates of the parameters which determine flux could be made prior to achieving steady state conditions. We developed a mathematical description of a static diffusion cell to use as a tool for estimating absorption parameters. Amount of chemical in receptor solution is simulated with the model to provide estimates of flux and diffusion. This tool has been applied to absorption of chemicals through a butyl rubber membrane and isolated rodent skin. This method of mathematical modeling is useful for getting information from *in vitro* diffusion studies prior to steady state.

872 *IN VITRO* PERCUTANEOUS ABSORPTION OF BENZIDINE IN COMPLEX CHEMICAL MIXTURES

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Humans are more likely to be exposed to chemical mixtures than to a single chemical. We define mixtures as consisting of a marker chemical and several other chemicals, each of which can have different mechanistic actions on dermal penetration and absorption of the marker chemical. Ten mixtures, consisting of a marker chemical (benzidine, B), a solvent (acetone, A or DMSO, D), a surfactant (0 or 10% sodium laurel sulfate, SLS), a vasodilator (0 or 180 mg methyl nicotinate, MNA), and a reducing agent (0 or 2% $SnCl_2$,) were studied. Isolated perfused porcine skin flaps (IPPSFs), which have proven to be a suitable *in vitro* model for assessing dermal absorption and toxicity, and flow-through diffusion cell systems were utilized. Data from the diffusion cell system was comparable to data from the IPPSFs when B + A and B + D mixtures were tested. For mixtures containing (1) DMSO only or acetone only or (2) solvents with SLS + MNA, benzidine absorption was enhanced when compared with other mixtures. Compared to other mixtures tested, $SnCl_2$ inhibited benzidine absorption independent of solvent tested. DMSO compared to acetone appears to enhance dermal penetration of benzidine for most mixtures. $SnCl_2$ also appears to inhibit benzidine penetration in DMSO mixtures containing SLS only, but not acetone mixtures. It is proposed that potential chemical-chemical interactions between benzidine and $SnCl_2$ may be inhibiting benzidine absorption and potential chemical-biological interactions between MNA + SLS and skin may be enhancing benzidine absorption. Supported by NIEHS grant ES 00044.

873 WATER PERMEATION BARRIER IN ISOLATED CUTANEOUS NEWBORN RAT EPIDERMIS

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Abnormal water barrier function occurs in irritated skin and certain cutaneous diseases. Methods have been compared for separating the epidermis - site of the barrier - from the whole skin without disturbing barrier function. The epidermis was separated from newborn rat skin by : (a) exposure to 10% trypsin, 4°C, 16 hr, (b) exposure to 0.2% dispase, 4°C, 16 hr, (c) heating for 50 sec in 55°C water, (d) or heating for 40 sec in 50°C water after the whole skin was kept at the air-liquid interface for one day at 35°C in complete MEM (In Vitro Cell Dev. Biol. 1995, 31, 283). Water permeation of the isolated epidermis was then measured immediately or after 3, 5, 8, and 10 days of maintenance at the air-liquid interface. The water permeation barrier constant (Kp) was 1.9 ± 0.9 cm/hr (n = 8) in intact rat skin. At 0 day of maintenance the Kp of the epidermis was 2.1 ± 0.9 after trypsin (n = 4), 3.8 ± 1.2 after dispase (n = 4), 4.3 ± 1.4 after immediate heating (n = 4), and 2.2 ± 0.7 cm/hr after culture and heating (n = 4). The dispase and heating methods disrupted the barrier to a greater extent than did the trypsin and culture-heating methods. The latter two methods allowed the Kp to be maintained for 8 days (Kp for trypsin 2.8 ± 0.9 and 2.5 ± 0.8 for culture-heating) when the medium was changed daily. Epidermis isolated by the trypsin or culture-heating techniques and maintained at the air-liquid interface can be used to study the mechanism of barrier function disruption by chemicals.

874 EVALUATION OF THE SKIN SENSITIZATION POTENTIAL OF WHITE MINERAL OIL, PETROLATUM AND GELLED WHITE OIL

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The skin sensitization potential of a National Formulary (NF) grade white mineral oil, Pennzoil's Penreco® Drakeol® 5, CAS No. 8042-47-5, and a Penreco® United States Pharmacopoeia (USP) grade white petrolatum, CAS No. 8009-03-8, was evaluated using a guinea pig skin sensitization assay, Beuhler method; and a gelled white oil was evaluated in a human repeated insult patch test (HRIPT). In the guinea pig assay, animals were treated with the test material at the same site, once a week for three weeks. After a two week rest period, animals were challenged at a contralateral virgin site on the back. The test material was considered a sensitizer if the mean irritation scores, the total number of animals with scores, and/or the total number of scores for the virgin test site in induced animals were appreciably greater than those for the naive or vehicle cotntrol groups. A mineral oil based gel

called Penreco® Geahlene® 750, which is mixture of over 90% of a NF grade white mineral oil and approximately 10% gellants consisting of saturated alkene copolymers, namely hydrogenated butylene / ethylene / styrene and hydrogenated ethylene / propylene / styrene copolymers, was tested in the HRIPT using the method of Marzulli and Maibach. All induction patches were applied at the same site on the back, three times a week for three weeks. Panelists were challenged two weeks after the last induction by application of patches to the dorsal aspect of the right or left upper arm. Reactions were scored 48 to 96 hours after application. Neither the white mineral oil, nor petrolatum produced a sensitization reaction in guinea pigs as judged by the reactions produced after challenge at a virgin site by a non-irritating dose. The white oil gel did not produce skin sensitization in human volunteers. These results support the use of these products in applications involving prolonged and repeated skin contact.

875 IMMUNOHISTOCHEMICAL COMPARISON OF INTEGRIN SUBUNITS α2, α3, AND β4 EXPRESSION IN HUMAN SKIN, PIG SKIN AND ISOLATED PERFUSED PORCINE SKIN FLAP

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Integrins α2β1, α3β1, and α6β4, are expressed in the epidermis, and play an important role in wound-healing and epidermal-dermal interaction. The isolated perfused porcine skin flap (IPPSF) has been shown to be an in vitro model for chemical induced-vesication. In order to determine whether the IPPSF could be utilized to study chemical vesication in skin mediated by integrins, the expression of integrins α2β1, α3β1, and α6β4 was studied in human skin (HS), pig skin (PS) and IPPSF using immunohistochemical techniques. Staining of α2β1 and α3β1 was located at the periphery of stratum basale cells of the epidermis in HS, PS and the IPPSF. However, α3 antibody outlined the keratinocytes in all epidermal layers of the IPPSF and PS. In HS, PS and the IPPSF, α6β4 showed a continuous linear labeling at the epidermal-dermal junction. The IPPSF and PS showed stronger immunoreactivity with the antibody against β4. The distribution of α6β4 in bis-2-chloroethyl sulfide (HD) induced blisters were also examined in the IPPSF. The α6β4 staining was discontinuous and exclusively located on the epidermal side of the blister. This study demonstrates that the IPPSF expresses similar integrins to that of PS, HS, and the distribution of α6β4 in IPPSF blisters caused by HD is similar to some human basement membrane blistering diseases. Therefore, PS and the IPPSF are suitable models to study the role of integrin in wound-healing and blistering diseases occurring at the epidermal-dermal junction. (Supported by USAMRDC-DAMD17-92-C-2071)

876 SYSTEMIC TOXICITY OF THE HEAVY FRACTION OF A BITUMEN UPGRADING PRODUCT IN RATS FOLLOWING SUBCHRONIC DERMAL EXPOSURE

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The systemic toxicity of the heavy fraction of a bitumen upgrading product (heavy gas oil II, BHGO) was investigated in Sprague Dawley rats following subchronic, dermal exposure. BHGO was applied daily on the skin of rats at doses of 8, 20, 50 or 125 mg/kg body weight for 13 weeks. The sham control group received normal saline while the positive control was given a coal liquefaction product (CLP) at 125 mg/kg body weight. Treatment with BHGO resulted in growth suppression starting at 8 mg/kg in females and 50 mg/kg in males. Relative liver weights were increased in animals of both sexes starting at the lowest dose. Similar effects on the growth rate and organ weight were shown in CLP treated animals, but the magnitude of the effects was comparable to that of 20 mg/kg BHGO. A dose-dependent elevation in serum cholesterol was seen starting at 8 mg/kg in both sexes. Anemia including decreased hemoglobin and red blood cells occurred in the medium and high-dose groups of both sexes. Treatment with BHGO resulted in moderate to severe histopathological changes in thymus and thyroid. Based on these data the no observable adverse effect level of BHGO was below 8.0 mg/kg body weight.

877 EFFECTS OF GREEN TEA POLYPHENOLS ON CULTURED NORMAL HUMAN KERATINOCYTES

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Green tea has demonstrated anti-tumor effects. The polyphenolic fraction isolated from green tea (GTP) has been shown to reduced ultraviolet-B and chemically induced cutaneous inflammation. The anti-inflammatory property of GTP led us to investigate the mechanism of action of GTP on cultured normal human keratinocytes. The effects of GTP was assessed by arachidonic acid release and proinflammatory cytokine mRNA regulation. Keratinocytes were labelled with ^{3}H arachidonic acid, irradiated with UVB and incubated with GTP. GTP demonstrated a reduced UV-induced arachidonic acid release. The effect of GTP on constitutive IL-1α mRNA expression was examined by reverse transcription-polymerase chain reaction (PCR). PCR results showed a down regulation of IL-1α message in a dose depended manner. Our findings suggest that the anti-inflammatory effects of GTP are in part due to reduced cell membrane damage and down regulation of IL-1α message in human keratinocytes.

878 SPECIFIC ANTAGONIST OF RETINOID TOXICITY IN MICE

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AGN 193109 is a potent antagonist of retinoic acid receptors *in vitro*. The goal of the present study was to determine if AGN 193109 could prevent and/or treat retinoid toxicity *in vivo*. Female hairless mice were treated once daily topically for 5 days with the retinoic acid receptor agonist (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl) propen-1-yl] benzoic acid (TTNPB) alone (0.072μmol/kg/d) or in combination with a 1, 4, or 16-fold molar excess of AGN 193109 and euthanized on day 8. TTNPB caused skin flaking, skin abrasion, and splenomegaly, and these effects were inhibited in a dose-dependent fashion by AGN 193109 co-treatment. To determine if topical AGN 193109 could prevent toxicity induced by an oral retinoid, mice were treated by gavage with TTNPB (0.75 μmol/kg/d) and topically with 0, 0.3, or 1.2 μmol/kg/d of AGN 193109 for 4 days. Oral TTNPB treatment caused skin irritation and weight loss, and these effects were inhibited by AGN 193109 co-treatment. To test the ability of AGN 193109 to treat preexisting retinoid toxicity, mice were pretreated topically on days 1-2 with TTNPB (0.72 μmol/kg/d), treated topically on days 3-5 with 0, 1.4, 7.2, or 22.5 μmol/kg/d of AGN 193109, and observed until day 8. TTNPB pretreatment caused severe weight loss and, in mice treated subsequently with vehicle, 60% mortality. AGN 193109 treatment at all dose levels accelerated recovery of body weight and prevented death in TTNPB-pretreated mice. These data show that AGN 193109 is an effective antagonist of retinoid toxicity and a potential retinoid antidote *in vivo*. AGN 193109 should also be a useful experimental tool for the study of retinoid biology.

879 ASSESSMENT OF CUTANEOUS SULFUR MUSTARD INJURY IN THE MOUSE EAR EDEMA MODEL

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Mustard gas (bis(2-chloroethyl)sulfide, BCES, HD) is a potent cutaneous vesicant that rapidly penetrates the skin causing gross and microscopic pathological changes in humans. No definitive animal model forms elevated fluid-filled blisters (bullae) like those seen in human skin, but skin from animals does develop erythema, edema and eventual separation of the epidermal-dermal junction to form microblisters which are consistent with blister formation in humans. Five dosages of BCES (0.08, 0.16, 0.32, 0.64 or 1.28 mg) in methylene chloride were applied to the inner surface of the right ears of male CD1 mice (n = 10/dosage). Methylene chloride only was applied to the left ears(vehicle control). At 6, 12, 18 and 24 hrs post-exposure, 8 mm dermal punch specimens were taken from the center of exposed and control ears and weighed to determine fluid accumulation (edema). Skin punches were then fixed in 10% formalin, embedded in paraffin and stained with hematoxylin–eosin (H&E) for histopathological evaluation. A two way ANOVA on the percent of control edema weight found a significant dose by time interaction. On microscopic examination, BCES caused separation of the epidermal-dermal junction (microblisters), and epidermal and basal cell layer necrosis which is consistent with vesicant injury. The incidence of microblister formation was greater for all dosages at 18 and 24 hrs post-exposure than for dosages at 6 and 12 hrs post-exposure. Dose and time related edema increases

and microscopic pathological changes in BCES exposed mouse ear skin support the use of this model for future studies involving medical countermeasures to cutaneous BCES injury.

880 *IN VIVO* PERCUTANEOUS ABSORPTION OF ACETOCHLOR IN RHESUS MONKEY: DOSE RESPONSE AND EXPOSURE RISK ASSESSMENT

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The percutaneous absorption of three topical dose levels of [^{14}C]-acetochlor in the rhesus monkey was done for exposure risk assessment. Topical doses were 30.7, 0.43 and 0.03 mg acetochlor in 40 µl commercial formulation and aqueous dilutions thereof, spread over 10 sq. cm. skin surface area. The dosing area was not covered. The dosed skin was washed with 50% soap (Ivory® liquid) and water after the 24 hours dosing period. An iv dose of 0.43 mg was also administered to determine the excretory kinetics of acetochlor. The same four monkeys were used for all dose administrations. Bioavailability was determined by radioactivity disposition in blood, urine and feces.

Percutaneous absorption was 23.3 ± 9.1 %, 17.6 ± 4.9% and 4.9 ± 1.4% for 0.03 mg, 0.43 mg and 30.7 mg doses, respectively. Assuming a constant state of absorption, the hourly exposure flux (µg/cm^2/hr) was 0.03 ± 0.01 (3 µg/cm^2); increasing the dose 15-fold (43 µg/cm^2) gave a 10-fold increase in flux to 0.3 µg/cm^2/hr, and increasing the dose 70-fold (3070 µg/cm^2) gave a 21-fold increase. The efficiency of absorption (percent) decreased with increased topical dose, but the amount (mass/flux) of acetochlor absorbed always increased with increased dose. Plasma levels of topical acetochlor at the high dose were detectable at one hour and continued at a somewhat steady level through the 24 hour dosing period. After the skin wash (24 hour) plasma levels decreased but were still detectable at the last 168 hour sampling period.

Acetochlor, recently EPA approved as a weed killer for corn crops, is carcinogenic; however, farmers will use half as much acetochlor per acre as other herbicides. The percutaneous absorption of acetochlor is equal to that of alachlor. Therefore, human exposure based on one-half usage suggests that human risk assessment should be one-half, all other factors being equal.

881 *IN VIVO* DERMAL ABSORPTION OF DICHLOROBENZENE, CHLOROPENTAFLUOROBENZENE AND TRI-DECAFLUOROIODOHEXANE IN FISCHER 344 RATS

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Dermal exposures to chemicals in the workplace may cause a systemic hazard if exposures are prolonged or spread over a large surface area. Dermal absorption kinetics of dichlorobenzene (DCB), chloropentafluorobenzene (CPFB) and tridecafluoroiodohexane (TFIH) were measured in male Fischer 344 rats during carefully controlled dermal exposures to the neat chemical. On the day before exposures the skin was carefully clipped of fur and a small glass cell was superglued to the middle of the rat's back. Blood concentrations were measured serially from an indwelling jugular cannula for up to four hours. Physiologically-based pharmacokinetic (PBPK) models for each chemical were used to estimate real-time flux and permeability coefficients for each of these six carbon chemicals. Permeability coefficients varied by over an order of magnitude. As judged by histology, these chemicals all affected the skin to some extent during the exposures. PBPK models suggested that the decrease in blood concentrations during the exposures was due to a decrease in rate of absorption rather than a decrease in concentration on the surface. These studies demonstrate that PBPK models can be used as a tool to understand changes in the absorption process. (Supported by Air Force Office of Scientific Research)

882 13-WEEK DERMAL TOXICITY & CELL PROLIFERATION STUDY OF A HYDROQUINONE CREAM

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Male and female F-344 rats were topically treated with 0, 2.0, 3.5, or 5.0% hydroquinone (HQ) in an oil-in-water emulsion cream for 13 weeks (5 d/wk). The formulation was representative of OTC preparations sold in the USA and EU. Animals were observed for clinical signs of toxicity and dermal irritation. Body weights, feed consumption, and water consumption were measured at least weekly. No HQ-related signs of toxicity were noted. Erythema was seen at the application site but it abated on the weekends. No differences in body weight were noted although feed consumption values by the 3.5 and 5.0% male rats were lower than by controls. No differences in feed consumption by female rats were seen, and water consumption was comparable among groups. No biologically significant changes in hematology or red blood cell morphology were noted. Increases in AST, ALT, SDH activities and total protein concentrations may be related to bandaging since an occasional animal in all groups, including controls, had high activities. Urinalysis did not indicate any kidney damage. No differences in organ weight were seen, and there were no HQ-related histopathologic lesions. Cell proliferation in the kidneys was evaluated after 3, 6, and 13 weeks of treatment using BrdU labeling, but no changes indicative of sustained cell proliferation were seen. Thus, the male F-344 rat-specific kidney effects observed in previous studies after oral HQ dosing are absent when exposure is dermal.

882a ROLE OF A TOPICARE DELIVERY COMPOUND® (POLYOPREPOLYMER-2) IN THE REOCCURRENCE OF MUCOCUTANEUS INFECTIONS

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Mucocutaneous infections remain an important clinical problem. New formulations, as well as new substances may provide therapeutic improvement. The ultimate goal of this study was to determine how a new excipient may influence the efficacy of topical formulations. Three vaginal cream formulations containing 1% clotrimazole as the active antifungal ingredient as well as a control formulation which contained no clotrimazole were tested. Two of the test formulations contained a Polyoprepolymer-2 - a mixture of liquid polymers, designed to hold skin care agents at targeted levels on and in the upper layers of the epithelial tissues. The third formulation was a commercially available antifungal cream. The efficacy of these four formulations was compared in a blinded study using an animal model (n = 36) of *Candida Albicans* vaginal infection (Larsen and Galask, 29:863, 1984). None of the formulations showed efficacy in terms of negative cultures after 1 dose of a product. All three showed efficacy after the second dose. Based on the rapidity of attaining negative cultures, the average number of days spent with positive cultures and the mycologic counts among animals there were no statistical differences between the three formulations used. Addition of Polyoprepolymer-2 to a 1% clotrimazole cream formulation reduced by 20% the reoccurrence of the vaginal candidiasis in experimental animals. This may be due to the reservoir effect of Polyoprepolymer-2, thereby increasing retention of the active substance in the upper layers of the epithelia.

883 THE EFFECT OF LEAD ON NEURITOGENESIS

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Despite several decades of research on the neurotoxic mechanisms of lead and its continued prominence as a major environmental and occupational health hazard, its toxic actions in the nervous system are still unknown. The Center for Disease Control (CDC) concluded that blood lead levels in excess of 10 µ/dl (0.483 µmol/l) are associated with increased risks of neurotoxicity. The processes promoting neuritogenesis, neurite outgrowth, and synaptogenesis present a variety of potential molecular targets for lead. Previous findings in our laboratory by Ndifor demonstrated a direct positive correlation between lead levels and the rate of formation of neurite processes in PC-12 cells. In an attempt to find out if these observed effects are cell line specific, we have looked at the effect of lead on neuritogenesis in mouse neuroblastoma cells. Mouse neuroblastoma cells (NB41A3) obtained from ATCC were used to investigate the effect of lead on nerve cell differentiation, the formation of neurites. Cells were grown, subcultured, and plated to be exposed at various concentrations of lead. Nerve growth factor was used at a concentration of 50 ng/ml to enhance neuritic extensions. Neuritogenesis was visually determined with an inverted light microscope. A preliminary 24 hour lead experiment indicated that lead decreased the number of cells which differentiated, underwent neuritogenesis, at exposure doses of 0.1, 1, 10, 25, and 50µg/dl, compared to the control which had no lead added. Studies are currently underway to observe leads affect on the length of these neurite extensions. (supported by ATSDR Grant #U50/ATU39894802)

884 UPTAKE OF INORGANIC MERCURY BY LLC-PK_1 CELLS

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The effects of cell density, temperature, sodium ions, a metabolic inhibitor, halide ions, and copper (Cu) on the uptake of inorganic mercury (Hg) by LLC-PK_1 cells were investigated. The cells were cultured for 3–12 days, then incubated at 37° or 4°C for 30 min in Hanks' balanced salt solution (HBSS) containing 1 μM Hg. After the incubation, the cells were washed with PBS containing BAL to remove free and surface-bound Hg. Hg accumulation at 37° or 4°C decreased steadily as the cells reached confluence (day 7 or 8). The accumulation at 4°C was significantly lower than at 37° C irrespective of the days in culture. Pretreatment of the confluent cells (day 8) with a metabolic inhibitor, carbonylcyanide *p*-(trifluoromethoxy)-phenylhydrazone, decreased Hg accumulation 62%. Furthermore, replacement of NaCl in HBSS with choline chloride or KCl decreased Hg accumulation about 50%. In comparison, NaBr or NaI increased Hg uptake 53 and 126%, respectively, without affecting its efflux. Coincubation of Hg with 30 μM Cu increased Hg accumulation 123% by decreasing its efflux. The effects of halide ions and Cu were not altered by pretreatment of the cells with the metabolic inhibitor. The results of the present study agree with our previous reports of Hg and Cd uptakes by rat renal cortical epithelial cells and LLC-PK_1 cells, with the exception that while Cu decreased Hg efflux it competitively inhibited the uptake of Cd. These results suggest that (a) an increase in cell density restricts the uptake of Hg from the basolateral membrane due to the formation of tight junctions, (b) a part of the Hg is actively taken up from the brush border membrane through energy- and Na^+-dependent processes, (c) Br^- and I^- increase the passive transport of Hg without affecting its efflux, and (d) Cu increases Hg accumulation by decreasing Hg efflux from the cells.

885 CADMIUM UPTAKE BY AN HEPATIC HUMAN CELL LINE (WRL-68 CELLS)

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The mechanism of entrance of non essential toxic elements ocurrs probably through processes that exit for the essential metals. The objective of the present study was to investigate the mechanism of Cd uptake by an hepatic human cell line (WRL-68 cells). The accumulation of Cd was a non-saturable process that was biphasic over time. The initial rapid phase occurred during the first minute, followed by a sustained and slow accumulation during the 30 min of the determination. The presence of potassium cyanide did not cause any change in the uptake of Cd, suggesting that is not an active process. Low temperature (4°C) decreased the accumulation of the metal by 52% with 10μM of $CdCl_2$. N-ethyl-maleimide (NEM) inhibited ^{109}Cd accumulation by 34%. The Ca channel blockers caused significant decrease in the accumulation of Cd, 37% with nifedipine and 47% with verapamil. The results presented here indicate that the initial rapid uptake of Cd has characteristics of mediated transport, with no indication of the participation of an active process. Nearly, one third of the Cd entered WRL-68 cells through processes involving SH ligands. The calcium channels blockers had significant effect on Cd accumulation, suggesting that part of the metal enters the cell through the calcium channels.

886 LEAD IMPAIRS THE METABOLISM OF 25-HYDROXY-CHOLECALCIFEROL IN CULTURED RHESUS MONKEY KIDNEY CELLS

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25-hydroxycholecalciferol is the inactive precursor to 1,25 dihydroxycholecalciferol, the active form of vitamin D_3. The activation of 25-hydroxycholecalciferol primarily occurs in kidney proximal tubule cells. Lead intoxication has been shown to decrease serum 1,25 dihydroxyvitamin D_3 levels but with normal 25-hydroxyvitamin D_3 levels in children. To explore possible mechanisms by which lead exerts this effect, cultures of LLC-MK_2 cells were grown to confluency in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, then exposed to lead (0–30 μM) for 24 or 48 hours. Cultures were then incubated in serum-and lead-free medium with 50 nM 25-hydroxycholecalciferol for 1–3 hours, extracted for vitamin D metabolites and analyzed by HPLC. Lead exposure decreased the metabolism of 25-hydroxycholecalciferol in a concentration and time dependent manner. Our results also suggest that the vitamin D_3 metabolite profile (which includes 1,25, 24,25, and 25,26 dihydroxy forms) is changed in a complex fashion by lead. These data provide the first direct experimental evidence linking lead toxicity to inhibition of 25-hydroxyvitamin D_3 1 α-hydroxylase. Supported by NIEHS R104040.

887 EFFECT OF HEAVY METALS ON CHONDROGENIC DIFFERENTIATION OF EMBRYONIC CHICK LIMB CELLS

A Kanti and M A Smith. *Environmental Health Sciences, University of Georgia, Athens, GA.* Sponsor: P L Williams

Despite evidence that environmental contaminants affect growth and development and that good *in vitro* models exist for investigating normal cartilage development and bone formation (Puzas et al, 1992), there is very little information on the effects of environmental pollutants on these systems. Five heavy metals (As, Cr, Cd, Hg, Pb) were investigated for their effects on chondrogenesis in an *in vitro* chick limb micromass culture system. Confluent monolayer cultures were prepared from chick distal wing tips (stage 25; Hamburger and Hamilton, 1951) and incubated for 96 hours, fixed and stained with Alcian green followed by Crystal Violet. Photomicrographs show qualitative differences in staining intensity between each metal and control cultures. Proteoglycan (PG) synthesis as measured by Alcian green staining and cell proliferation (CP) as measured by Crystal Violet staining were determined spectrophometrically. Dose response experiments show that low doses (0.5 μg/ml) do not affect proteoglycan syntheses ($P>0.05$), whereas higher doses (As, Cd, Hg = 5 μg/ml; Cr and Pb = 50 μg/ml) show a decrease in both CP and PG syntheses ($P<0.05$). In temporal experiments a decrease in PG syntheses and CP was seen by 72 hours of culture. This suggests that heavy metals affect chondrogenic differentiation in chick limb bud cultures. Studies are underway to determine whether heavy metals affect DNA synthesis and repair. These results have significant implications in the use of the chick limb micromass culture system as a model system to study the teratogenic effects of metals and other compounds found in the environment on developing limbs.

888 EFFECTS OF PHYSICOCHEMICAL FORMS OF Ni COMPOUNDS ON CYTOSKELETAL ORGANIZATION AND CELLULAR THIOL CONTENT IN 3T3 CELLS

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The biological potency of Ni compounds depends on their physicochemical forms such that crystalline NiS exhibits carcinogenic and transforming activities whereas amorphous NiS is inactive. To further understand the mechanism of nickel -induced cell injury we have studied effects of crystalline and amorphous NiS on cytoskeletal organization and cellular thiol content in 3T3 cells. Exposure of cells to crystalline NiS resulted in a dose-dependent accumulation of particles in the perinuclear area forming a honeycomb-like structure which did not occur in amorphous NiS or $NiCl_2$ treated cells. Furthermore, crystalline, but not amorphous, NiS also induced the appearance of large microtubule (MT) bundles in the cell center area with microfilaments redistributed to the periphery, similar to those induced by $NiCl_2$. Antibody specific for acetylated tubulin recognized these bundled MT in crystalline NiS as well as $NiCl_2$-treated cells, indicating that crystalline NiS also stimulated α-tubulin acetylation, a marker for stabilized MT. In contrast, amorphous NiS-treated cells contained very little acetylated tubulin distributed in a few short, brightly-stained MT in the MT organizing center (centrosome) area. Crystalline NiS was also more potent than amorphous NiS in decreasing the levels of cytoskeletal and cytosolic protein sulfhydryls (PSH) and total cellular glutathione, which is important in maintaining "normal" levels of PSH. Cytoskeletal PSH in turn plays an important role in modulating MT integrity. Positive correlation between cytoskeletal injury (acetylated tubulin and MT bundling) and genotoxicity of crystalline NiS, but not amorphous NiS, suggests that MT perturbation may be an integral part of the mechanism of crystalline NiS carcinogenicity. (Supported by NIH RO1-ESO3543)

889 EFFECT OF LEAD ON COPROPORPHYRINOGEN OXIDASE IN HEPG2 CELL LINE.

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The objetive of this work was to study the effect of lead on the synthesis of porphyrins and to characterize the effect of this metal on the coproporphyrinogen oxidase activity taking as a model the HepG2 cell line. Cells were

treated with 150μM of δ-aminolevulinic acid (ALA) during 24h in order to accumulate porphyrins in the culture media and within the cells. Without the addition of ALA no prophyrins could be detected in HepG2 cells. 0.5, 1, 2.5 and 5 μM of lead acetate were used to evaluate the effect of lead on porphyrin synthesis. ALA and lead acetate were added 24h before the assay was performed. The highest concentration (twice the control value) of coproporphyrin in the culture media was obtained with lead acetate 1 μM, while protoporphyrin increased in the same extent, but within the cells, in presence of the same lead concentration. No detectable change was observed for uroporphyrin concentration. We observed a 36% decrease in coproporphyrinogen oxidase activity in presence of 0.5μM of lead acetate. Our results indicate that lead inhibited coproporphyrinogen oxidase activity that could be used as a useful biomarker of lead exposition.
This work was supported by **CONACYT 4307-M9406.**

890 TRANSPORT AND TOXICITY OF MERCURY-ALBUMIN COMPLEX IN THE ISOLATED-PERFUSED PROXIMAL TUBULE OF THE RABBIT KIDNEY

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The transepithelial lumen-to-bath (absorption) and bath-to-lumen (secretion) fluxes, cellular uptake, and toxicity of inorganic mercury in complexes with bovine albumin were studied in the S1, S2, and S3 segments of the proximal tubule. Toxicity of inorganic mercury was evaluated by histopathological inspection of the perfused tubule, uptake of the vital dye FD&C green, and by the transepithelial leak of the volume marker ^{3}H-L-glucose. When 20 μM inorganic mercury (^{203}Hg++) plus 120 μM albumin (fraction V) were simultaneously perfused through the lumen, or applied to the basolateral membrane, of any of the three segments of the proximal, none of the toxic effects normally induced by 20 μM inorganic mercury alone were detected during 50 minutes of perfusion. In addition, the cellular uptake of, and the lumen-to-bath and bath-to-lumen fluxes of inorganic mercury were not statistically greater than zero. Thus it appears that inorganic mercury formed complexes with albumin that were not transported along any of the segments of the proximal tubule to any measurable degree. In addition, it appears that the formation of these complexes prevents the toxicity of the inorganic mercury from occurring.

891 TRANSPORT OF CADMIUM THROUGH THE APICAL MEMBRANE OF LLC-PK$_1$ CELLS

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The effect of pH on the uptake and efflux of Cd through the brush border membrane was investigated in LLC-PK$_1$ cells and the effects of pH and Zn on Cd transport were investigated in brush border membrane vesicles (BBMV) isolated from these cells by Mg precipitation technique. The cells were cultured for 7 days to reach confluence and were incubated for 30 min in pH 5.5, 6.4 or 7.4 phosphate buffer containing 1 μM Cd. Upon termination of the incubation the cells were washed with PBS containing 2 mM EGTA to remove surface-bound Cd. The efflux of Cd into the phosphate buffers of different pH was measured in Cd-loaded cells (10 min incubation with 1 μM Cd at pH 7.4). The transport of Cd into BBMV was initiated by mixing 40 μl of BBMV preequilibrated at pH 5.5 or 7.5 with 200 μl of the incubation medium containing Cd at pH 5.5 or 7.5. EGTA was added to the stop and washing solutions to remove surface bound Cd and the transport of Cd was investigated by rapid filtration technique. A change of pH from 7.4 to 5.5 decreased cellular Cd accumulation 57% while increasing Cd efflux 11%. Since the initial transport of Cd into BBMV was stimulated by H^+ gradient, the increase in Cd efflux appears to be responsible for the decreased Cd accumulation by the LLC-PK$_1$ cells. Coincubation of the BBMV with Zn decreased Cd uptake in a concentration-dependent and competitive manner. Kinetic analysis of Cd transport suggested that Cd is transported through the brush border membrane not only via simple diffusion but also via carrier-mediated processes that are competitively inhibited by Zn. These results using BBMV are in agreement with our previous results of Cd uptake obtained from studies in monolayers of rat renal cortical epithelial cells and LLC-PK$_1$ cells.

892 EXPRESSION OF HSP27 CORRELATES WITH RESISTANCE TO METAL TOXICITY IN MOUSE EMBRYONIC STEM CELLS TRANSFECTED WITH SENSE OR ANTISENSE HSP27 cDNA

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Embryonic stem (ES) cells were transfected with the protein-coding region of rat HSP27 cDNA placed in sense or antisense orientation in vector pcDNA3 under the control of the constitutive cytomegalovirus (CMV) promoter. Compared with non-transfected ES cells, five sense HSP27 cDNA-transfected ES cell clones displayed up to 4-fold increases in expression of HSP27 mRNA and up to 6-fold increases in expression of HSP27 protein, whereas four antisense HSP27 cDNA-transfected ES cell lines exhibited synthesis of antisense HSP27 mRNA and a 50%-85% reduction of HSP27 protein expression. Compared to the parental ES cell lines, all ES cell lines constitutively overexpressing HSP27 were resistant to killing by $CdCl_2$, $HgCl_2$, and cis-platinum(II)-diammine dichloride, as well as by sodium arsenite and heat. In contrast, all ES cell lines expressing reduced HSP27 were more sensitive to metal toxicity and heat, as compared to non-transfected ES cells or ES cells transfected with the vector lacking any HSP27 sequence. The relative toxicities of the tested metals to ES cells were cis-platinum(II)-diammine-dichloride > sodium-arsenite > $HgCl_2$ > $CdCl_2$. Protection of ES cells against metal or heat toxicity was positively correlated with the level of HSP27 expression. These data confirm in ES cells previous reports of the ability of HSP27 to protect other cell types against heat, and show that the mechanism by which HSP27 protects eucaryotic cells operates as well to protect cells against the toxic effects of several metals. Supported by NIH grant ES06265 to MJW.

893 COMPARISON OF ARSINE AND ARSENITE TOXICITY IN CELLS REPRESENTING BLOOD, LUNG, LIVER, AND MUSCLE

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Arsine, or arsenic trihydride, is a highly toxic gaseous form of arsenic. Human whole blood, L132 cells (a human embryonic lung epithelial cell line), rat primary hepatocytes, and neonatal rat cardiac myocytes were treated with arsine and toxicity was determined by the release of LDH and K+ leakage. In blood, 1 mM arsine caused significant toxicity. At 60 minutes, 20.3±4.8 % of total LDH activity was released; by 120 minutes 47.6±7.1 % of total LDH was released. LDH release from blood treated with 1mM arsenite remained at control levels (1–2%). In L132 cells treated with up to 2.5mM arsine, LDH release remained at control levels (3–7%) at all points tested. Treatment with 2.5mM sodium arsenite produced increasing toxicity, with 21% of total LDH released by 6 hours. In rat primary hepatocytes, both arsine and arsenite caused toxicity (68.3±24.5 and 95.5±2.1 % of total LDH released at 3 hours, respectively). In rat primary cardiac myocytes, LDH release remained at control levels in arsine and arsenite treated cells (1–4%). Potassium leakage was used as another measure of toxicity. In blood, although LDH release was not observed until 1 hour, K+ leakage was observed as early as 5 minutes. Intracellular K+ concentration fell from 20 umol/g hemoglobin to 7.5 umol/g hemoglobin within 30 minutes. In other cell types, K+ leakage reflected LDH release. Hsp 70 induction was determined in hepatocytes treated with arsine or arsenite by Western blotting. Arsenite, but not arsine, caused induction of hsp 70. We conclude 1) that arsine and arsenite toxicity is cell type specific and 2) that arsine is not toxic by conversion to arsenite. This work was supported by NIH grants ES6644 and ES6694.

894 METHYLMERCURY (MeHg) MAY UTILIZE VOLTAGE-OPERATED CHANNELS FOR Ca^{2+} INFLUX AND/OR MeHg ENTRY INTO CEREBELLAR GRANULE CELLS

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MeHg causes preferential degeneration of cerebellar granule cells. We have hypothesized that MeHg alters intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), leading to a cascade of events culminating in cell damage and death. In rat cerebellar granule cells in primary culture, a large component of the MeHg-induced increase in $[Ca^{2+}]_i$ is due to influx of extracellular Ca^{2+}. To examine possible route(s) for Ca^{2+} influx, cultures of rat cerebellar granule cells (7-10 DIV) were exposed *in vitro* to 0.5 μM MeHg in the presence or absence of channel inhibitors. Ni^{2+} (1 μM), a non-selective inhibitor of Ca^{2+} channels,

effectively blocked 73% of a K^+-stimulated rise in $[Ca^{2+}]_i$, but had no effect on the $[Ca^{2+}]_i$ increase following MeHg exposure. Conversely, 1 μM ω-Conotoxin-MVIIC, which inhibits Q- and R-type Ca^{2+} channels, delayed the MeHg-induced elevation in $[Ca^{2+}]_i$. This occurred despite only a 38% reduction in the K^+-stimulated increase in $[Ca^{2+}]]_i$ by this agent. A previous study indicated that 1 μM nifedipine, a blocker of voltage-operated L-type Ca^{2+} channels, delayed the $[Ca^{2+}]_i$ rise although not significantly, whereas 1 μM tetrodotoxin, a voltage-dependent Na^+ channel blocker, did not alter the time-to-onset of MeHg-induced $[Ca^{2+}]_i$ elevations. Thus, MeHg may activate multiple voltage-operated Ca^{2+} channels for Ca^{2+} influx and/or MeHg entry. The number and variety of voltage-operated Ca^{2+} channels in cerebellar granule cells may contribute to their heightened sensitivity to MeHg exposure. Supported by NIH grants R01-ES03299, T32-ES07255-05 and T32-NS07279-08.

895 THE ACUTE EFFECT OF LEAD ACETATE ON GLUCOCORTICOID RECEPTOR BINDING IN C6 GLIOMA CELLS

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Specific cellular sites of action of the environmental pollutant, lead, have not been completely defined in the central nervous system (CNS). The present investigations were conducted to test the hypothesis that lead exposure perturbs glucocorticoid-mediated effects in CNS hormonal target tissues. The C6 glioma cell line was selected as the model for these investigations. Cells were treated with 1-30 μM lead acetate for 24 or 48 hr in absence or presence of the glucocorticoid dexamethasone (DEX). Lead concentration-dependently altered binding of [^{3}H]DEX to the cytosolic glucocorticoid receptor in whole cell binding assays. Treatment times (24-48 hr) and concentrations of lead acetate (1-30 μM) did not significantly alter total cell numbers, [^{3}H]thymidine incorporation or trypan blue exclusion. Glucocorticoid receptor binding studies yielded a Kd = 10.5 ± 0.5 nM and a Bmax = 486 ± 27 fmol/mg protein in untreated cells versus a Kd = 23.1 ± 2.6 nM and Bmax = 472 ± 35 fmol/mg protein in cells exposed to 10 μM lead acetate for 24 hr. PKC activity assays on C6 cells treated with sodium or lead acetate and DEX indicated that both lead and DEX induce a redistribution of PKC from the soluble to the particulate fraction, but lead-treated cells contained 52% less total PKC activity than sodium acetate treated cells. Phorbol myristate acetate did not potentiate binding indicating that atypical PKC isozymes may be involved. Together, these results suggest that acute exposure of C6 cells to lead may inhibit processes involved in glucocorticoid-mediated signal transduction events within CNS hormonal target cells. (Supported by ATSDR and NIH 03320).

896 HYDROGEN PEROXIDE (H_2O_2) GENERATION BY SELENITE IN MOUSE ERYTHROCYTES AND ITS ROLE IN HEMOLYSIS

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We have recently demonstrated the active oxygen generation by the reaction of selenite with sulfhydryl compounds such as reduced glutathione *in vitro*. Two types of mouse erythrocytes (MRBC), MRBC from C57BL mice and those from C3H mice, were used to investigate the possibility of H_2O_2 generation and its role in selenite cytotoxicity. Total sulfhydryl content in C3H-MRBC is higher than that in C57BL-MRBC. One percent suspension of MRBC was incubated with various concentrations of sodium selenite at 37°C in the presence or absence of aminotriazole (AT) which specifically inactivates catalase only in the presence of H_2O_2. An aliquot was taken after 10 min incubation and assayed for catalase activity. The rate of hemolysis was determined after 90 min incubation by measuring hemoglobin concentration in the supernatant of MRBC suspension. Catalase activity was decreased substantially both in C3H-MRBC and C57BL-MRBC suspensions by the addition of selenite in the presence of AT. The extent of CL inactivation in the presence of AT as well as hemolysis caused by selenite was greater in C3H-MRBC than in C57BL-MRBC. These results suggest that selenite generates H_2O_2 in MRBC suspension dependently on sulfhydryl content, and that the generated H_2O_2 contributes to hemolysis.

897 INTERACTION OF CADMIUM WITH A CALCIUM-BINDING POLYPEPTIDE ANALOG OF E-CADHERIN

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Many effects of cadmium Cd^{2+} *in vivo* appear to involve increases in the permeability of various epithelial surfaces. However, the mechanisms by which these changes in permeability occur have yet to be elucidated. Studies from our laboratory have shown that Cd^{2+} can selectively damage the tight junctions between cells of the renal epithelial cell line, LLC-PK_1. Recently, we have obtained evidence suggesting that this effect may involve the interaction of Cd^{2+} with E-cadherin, a Ca^{2+}-dependent cell adhesion molecule that is localized at the adhering junctions of epithelial cells. To determine whether or not Cd^{2+} might bind directly to the E-cadherin molecule, we studied the binding of Cd^{2+} to a recombinant 145-residue polypeptide that corresponds to the N-terminal sequence of mouse E-cadherin. This polypeptide (termed E-CAD1) was a generous gift from Dr. Mitsuhiko Ikura, of the University of Toronto. The binding of Cd^{2+} to E-CAD1 was evaluated by using an equilibrium microdialysis technique and the radioactive isotope $^{109}Cd^{2+}$. The results showed that Cd^{2+} could, in fact, bind to E-CAD1. The binding was saturable, with a maximum of one Cd^{2+} binding site per E-CAD1 molecule. The apparent dissociation constant (K_d) for the binding was about 20 μM, a concentration similar to that which has been shown to disrupt the junctions between LLC-PK_1 cells. Other results showed that the binding of Cd^{2+} was greatly reduced when excess Ca^{2+} (1 or 10 mM) was included in the dialysis solution. These results suggest that Cd^{2+} can interact with the Ca^{2+} binding site on the E-CAD1 molecule, and they provide additional support for the hypothesis that E-cadherin might be a target for Cd^{2+} toxicity. (Supported by NIH Grant ESO6478).

898 Ni^{2+} ENHANCEMENT OF MICROTUBULE ASSEMBLY *IN VITRO* IS DEPENDENT ON GTP FUNCTION

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Microtubule (MT) assembly *in vitro* is accompanied by GTP hydrolysis and the exchange of GDP for GTP at the E-site of tubulin and enhanced by Ni^{2+}. To further understand the mechanism of Ni^{2+}-induced MT injury we have studied the effects of Ni^{2+}/GTP interaction on enhancing MT assembly *in vitro* as monitored by a turbidity assay at $A350_{nm}$ at 27°C using twice-cycled bovine brain MT proteins (3 mg/ml) that contained mainly tubulin and minor amounts of MT associated proteins with intrinsic Mg^{2+} and EGTA each at 162 μM. MT assembly was initiated by addition of exogenous GTP and progressed in a GTP dose-dependent manner. The minimal and optimal doses of exogenous GTP required for MT assembly under these conditions were 15.6 and 500 μM, respectively. Proportional substitutions of GTP with $NiCl_2$ while keeping the sum of [GTP] and [Ni^{2+}] constant at 500 μM, enhanced MT assembly by decreasing the initial lag, increasing the elongation rate, and shortening the time to reach the final plateau. However, when 97% of GTP was replaced with Ni^{2+} MT assembly was largely halted. Electron microscopic (EM) examination showed that MT assembled in the presence of Ni^{2+} appeared shorter, more numerous and more resistant to Ca^{2+} depolymerization than those obtained with 500 μM GTP without Ni^{2+}. Notably, at 500 μM Ni^{2+} but in the absence of exogenous GTP, no assembled MT were observed under EM although the turbidity of the reaction mixture still increased. In addition, the critical concentration of MT proteins required for assembly was considerably decreased under conditions of Ni^{2+} replacement of GTP. These results suggest that Ni^{2+} enhancement of MT assembly *in vitro* is modulated by its interaction with GTP. (Supported by NIH RO1-ES03543)

899 EFFECT OF NON-TOXIC MERCURY, ZINC, OR CADMIUM PRETREATMENT ON THE CAPACITY OF HUMAN MONOCYTES TO UNDERGO LIPOPOLYSACCHARIDE-INDUCED ACTIVATION

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Metal salts can inhibit cell activity through direct toxicity to critical cellular molecules and structures. On the other hand, they can also change cell behaviour through induction of specific genes (including genes encoding members of the metallothionein gene family). Therefore, metals may affect cell functions by both signalling and toxic events. To explore the former possibility, we measured the ability of low, non-toxic metal pretreatment to alter immune cell behaviour. We previously reported that pretreatment of human monocytes with zinc induces metallothionein gene expression and

alters their capacity to undergo a bacterial lipopolysaccharide (LPS)-induced respiratory burst[3]. We report here that Cd and Hg, at concentrations that exert no discernible toxicity (as measured by both metabolic and growth efficiency assays), have a similar inhibitory effect on activation of a human monocytic leukemia cell line (THP-1). 1 μM $CdCl_2$, 20-40 μM $ZnCl_2$, or 2 μM $HgCl_2$ pretreatment for 20 hours induced MT-2 mRNA and total MT protein accumulation and had no effect on proliferation potential or metabolic activity, but significantly inhibited the ability of subsequent LPS to induce both an oxidative burst and MT-2 and IL-1β mRNA accumulation. The phenomenon of metal-induced suppression of monocyte activation, at metal concentrations that have no effect on cell viability, has important implications for assessment of acceptable levels of human exposure to cadmium, zinc, and mercury.

[3]MEI Leibbrandt and J. Koropatnick (1994). Toxicology and Applied Pharmacology 124, 72-81.

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900 LEAD/CYTOKINEHEPATOTOXICITYINVOLVESOXIDATIVE DNA DAMAGE

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Lead, a known hepatomitogen, synergizes with tumor necrosis factor-α (TNFα) to enhance liver injury *in vivo*. However, the mechanism for this hepatotoxicity remains unknown. To examine the mechanism(s) of this interaction, primary cultures of mouse hepatocytes were treated with combinations of Pb and the cytokines TNFα and interferon-γ (IFNγ). No significant cytotoxicity was observed with the concentrations of Pb used. However, in combination with TNFα(0.1μg/ml) + IFNγ(100U/ml), Pb(50μM) demonstrated significant cytotoxicity versus control or cytokines alone; 70.5 ± 9.8% versus 25.1 ± 4.9% and 31.0 ± 5.8%, respectively. Pb(50μM) alone significantly increased DNA synthesis, as indexed by ^{3}H-thymidine incorporation and cellular DNA content, *in vitro*. DNA fragmentation was evident in cells treated with cytokines + Pb (50μM). Both increased cytotoxicity and DNA fragmentation were blocked by treatment with antioxidants. These data suggests that the cytotoxic interaction between Pb and cytokines occurs at the level of DNA. To futher investigate this hypothesis, we examined hepatocytes cultured at a low cell density. These cells spontaneously undergo DNA synthesis. Under these conditions, the cytokine combination of TNFα(0.1μg/ml) + IFNγ(100U/ml) caused significant cytotoxicity versus control cultures; 51.5 ± 6.6% versus 24.0 ± 3.6%, respectively. This cytotoxicity was also blocked by antioxidant treatment. These results indicate that hepatocytes which are actively synthesizing DNA are sensitized to an oxidative DNA damage event which is induced by cytokine treatment. Supported by NIH grant DK44755.

901 EFFECTS OF LEAD ON CATECHOLAMINE UPTAKE AND RELEASE IN PC12 CELLS

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Lead has been reported to modulate catecholamine uptake and release in mammalian cell lines. It has, for example, been shown to enhance norepinephrine release in permeabilized chromaffin cells in a process associated with its effects on protein kinase C. We have studied the effects of lead (Pb^{2+}) on the release of catecholamines into the culture media and the uptake of radiolabelled dopamine (DA) and norepinephrine (NE) in PC12 cells. Cells were cultured for 24hrs in the presence of 4.826nM- 1.2065μM Pb^{2+} levels and the culture media was analysed via HPLC for catecholamines. Cells were also harvested, and incubated in the presence of Pb^{2+} and ^{3} H-NE or DA for 1hr to assess their uptake. Our results indicate that Pb^{2+} was a more potent inhibitor of NE than DA release in exposed cells. It however, had no effect on NE uptake within a concentration range of 0.4826nM to 4.826μM. Interestingly, DA uptake in the same model was enhanced in the presence of Pb^{2+}. The significance of Pb^{2+} as an uptake enhancer, and, as we have previously reported, an inhibitor of catecholamine biotransformation, both important processes in the termination of released neurotransmitter activity is discussed.

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902 TRANSFORMATION INDUCED BY EXPOSURE TO SODIUM ARSENITE IN CULTURED RAT LIVER CELLS ASSOCIATED WITH DNA HYPOMETHYLATION

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Arsenic compounds are known human carcinogens and have been linked to cancers at various sites, including liver. *In vitro*, trivalent arsenic (arsenite, As^{+3}) can induce transformation in several cell lines. Methylation is a very important aspect of As^{+3} metabolism in many cells, including hepatocytes, while DNA hypomethylation has been associated with hepatocarcinogenesis. These experiments examined the ability of chronic low dose As^{+3} to induce transformation in liver cells in order to develop a model to help define the molecular events that may occur in As^{+3}-induced carcinogenesis. TRL 1215 cells, a diploid, nontumorigenic, epithelial-like line derived from 10 day-old rat liver were used. The LC_{50} of sodium As^{+3} was 3.22 ± 0.14 μM in these cells. Cells were continuously exposed to non-toxic levels of As^{+3} (125, 250 and 500 nM) for up to 18 weeks. Culture in the presence of As^{+3} resulted in dose-related transformation starting at 8 weeks as assessed by a clear morphology change from epithelioid to fibroblast-like. In addition, the genomic DNA isolated from these cells appears to be undermethylated compared to control cells. Global DNA methylation was determined with DNA isolated from control and As^{+3}-treated (500 nM for 8 weeks) cells. Isolated DNA was digested with *Msp*I and its methyl-sensitive isoschizomer *Hpa*II, followed by Southern blot analysis using a rat satellite repeat genomic DNA probe (pCR 784). Results indicate that DNA from As^{+3}-treated cells was significantly undermethylated compared to control cells. These data indicate that As^{+3} can both induce transformation and can cause DNA hypomethylation in this liver cell line. TRL 1215 cells have previously been shown to be sensitive to transformation induced by DNA hypomethylating agents. Thus they may prove to be a valuable model for the study of molecular events in As^{+3} carcinogenesis.

903 *IN VITRO* EXPOSURE TO CADMIUM IN RAT L6 MYOBLASTS CAN RESULT IN BOTH ENHANCEMENT AND SUPPRESSION OF MALIGNANT PROGRESSION *IN VIVO*

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Cadmium (Cd), a carcinogenic metal in humans and rodents, has been shown to transform cells *in vitro*. The effects of Cd have been studied in different mammalian cell culture systems, and Cd has also been shown to induce proto-oncogene activation. Cd, in certain instances, can also be anticarcinogenic. In the present study, the ability of Cd to affect malignant transformation was systematically investigated in L6 cells. Cells were grown in monolayer culture with concentrations of either 0 or 0.5 μM $CdCl_2$. After 9 weeks, cells treated with Cd showed growth of large colonies in soft agar, while control cells showed no detectable growth. When injected sc into athymic nude mice, the Cd-treated cells rapidly gave rise to large masses resulting in high host mortality (9 dead/9 injected, 100%, by 7 weeks). These tumors were subsequently diagnosed as highly malignant sarcomas. Mice injected with untreated cells also developed tumors but of significantly smaller size and growth rate and associated with a lower host mortality (4/10, 40%, $p \leq 0.01$, by 7 weeks). In fact, tumors resulting from untreated cells averaged approximately one-half the size (e.g. maximum diameter 12.9 mm at 23 days) of those resulting from injection of Cd-treated cells (22.2 mm at 23 days). However, Northern blot analysis indicated there was no c-*myc* or c-*jun* activation associated with this Cd-induced transformation of L6 cells. Cells exposed to higher levels of Cd *in vitro* (1.0 μM) resulted in decreased tumor growth *in vivo* compared to control, possibly demonstrating the anticarcinogenic capabilities of Cd. Thus, cells exposed to low levels of Cd *in vitro* showed a very rapid malignant progression *in vivo*, while slightly higher Cd levels *in vitro* suppressed *in vivo* tumor growth, reinforcing the concept that Cd can have both carcinogenic and anticarcinogenic effects.

904 PRIOR EXPOSURE TO PROGESTERONE INCREASES THE CYTOTOXICITY OF CADMIUM DESPITE ENHANCEMENT OF METALLOTHIONEIN EXPRESSION IN CULTURED RAT LIVER CELLS

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The effects of cadmium (Cd), a highly toxic metal and a very effective hepatotoxin, can be altered by exposure to certain steroids. Previously, we found that *in vivo* pretreatment with progesterone (PRO) markedly increased Cd lethality in rats, apparently by enhancing Cd-induced hepatotoxicity. In this study we sought to extend and expand these results by employing *in vitro* systems. Thus the effects of PRO on Cd toxicity were assessed in cultured rat liver cells using a line derived from newborn rat liver (TRL 1215). Cells were exposed to various levels of PRO (0, 1, 10 and 100 μM) for 24 hrs followed by Cd (0, 1, 5, 10 and 50 μM; as $CdCl^2$) for an additional 24 hrs. Although PRO by itself was essentially non-toxic, PRO pretreatment resulted in a concentration-dependent increased sensitivity to Cd cytotoxicity, as assessed both by metabolic integrity (tetrazolium-based dye assay) and loss of intracellular enzymes (glutamic oxaloacetic transaminase). The effects of PRO on metallothionein (MT), an inducible metal-binding protein generally associated with Cd tolerance, were also measured. PRO pretreatment (100 μM) modestly increased in MT levels (2.7-fold), while Cd alone resulted in a 4.4-fold increase over control. PRO pretreatment followed by Cd caused an marked, 18-fold induction in MT synthesis, a level of induction that is generally associated with acquired tolerance to Cd. Furthermore, PRO had no effect on levels of glutathione (GSH), which are thought to be important in Cd detoxication prior to MT synthesis. PRO pretreatment had no effect on Cd accumulation during the initial 3 hrs of metal exposure. These results show that PRO pretreatment clearly exacerbates Cd toxicity at the cellular level in liver cells. This effect is probably not based in altered toxicokinetics and occurs despite a marked induction of MT synthesis. The mechanism by which PRO enhances Cd toxicity deserves further study.

905 SUBCHRONIC TOXICITY STUDY OF NICKEL SULFATE ($NiSO_4$) IN THE RAT

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Adult male Sprague-Dawley rats (10 rats/group/dose) were exposed to $NiSO_4$ at 0, 0.1, 0.5 and 1.0% via drinking water for 2 weeks. 4 rats died at 1.0% dose level at the end of 2 weeks. Urines were collected for 24 hrs at the end of 2 weeks and the animals were then sacrificed. Body weight gain and body weights were decreased in 0.5 and 1% groups. Hematocrit value was increased at 0.5 and 1.0% dose. Significant increases in organ weight ratios were seen in the kidneys, lungs, brain, testes and decrease in the liver. Plasma glucose and proteins were decreased in 1.0% group. Urine glucose was increased at 0.1 and 0.5% dose levels, urine volumes were decreased at all dose levels, and blood urea nitrogen was increased at 0.5 and 1.0% dose levels, indicating damage to the kidney. Measurements of bronchoalveolar lavage fluid indicated some damage to the lungs. Significant increases in percentages and absolute numbers of splenic lymphocytes CD4+, CD8+ and B cells as well as the ratios CD4/CD8 were observed at 0.1% dose level, but at 0.5% dose level, all these parameters were significantly decreased, indicating damage to the immune system. Histopathology of different organs are currently being studied. Thus $NiSO_4$ was capable of producing toxicity to certain organs and tissues under the exposure conditions studied. In the second series, male Sprague-Dawley rats were exposed to $NiSO_4$ at 0, 0.02, 0.05 and 0.1% via drinking water for 4 weeks. No changes in the body weight gain, body weights, and in organ weight ratios were seen after 4 weeks. Hematocrit value was decreased only at 0.1% dose level. Urine volume was decreased, but urine glucose increased at all dose levels without affecting plasma glucose. $NiSO_4$ induced significant stimulation of lymphocytes T and B at 0.1% dose level. Thus toxicity of $NiSO_4$ is different depending on the exposure conditions.

906 DEVELOPMENT OF FLUORESCENT MARKERS FOR ASSESSING CELL INJURY

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The development of sensitive and selective fluorescent markers provides new opportunities for assessing adverse effects of toxicants on cells, cellular organelles, tissue slices, and organotypic cultures. Markers have become available for studying cellular pH, a variety of critical ions, competency of cell membranes, and mitochondrial and lysosomal function among others. The initial lecture will review basic principles underlying the use of these markers and will focus on a number of well established and new fluorescent markers that are of particular interest to toxicologists. The presentations that follow will exemplify the application of such markers under specific culture conditions and will identify confounding factors and necessary controls for interpretation of the outcomes. Workshop participants will discuss the utilization of fluorescent dyes in combination with confocal and traditional fluorescence microscopy in isolated cells and cell cultures, tissue slices, organotypic cultures, and in vivo. Participants will discuss the assessment of cellular pH and calcium, characterization of mitochondrial function and cell topology and the use of green fluorescent protein as a reporter gene in toxicology. The purpose of this workshop is to discuss the development of fluorescent markers, to highlight their use, to discuss their limitations and problems in interpretation, and to note new developments in this rapidly expanding area.

907 MOLECULAR BIOMARKERS IN ENVIRONMENTAL TOXICOLOGY

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In recent years, the great advances in our understanding of the mechanisms of action of toxicants and carcinogens has spurred the development of molecular biomarkers for use in risk assessment in individuals. These biomarkers extend across the spectrum from markers of internal dose, biologically effective dose, and host susceptibility factors to specific genetic targets. Experimental models have been critical for the development of these molecular biomarkers and these studies are providing valuable data for extrapolation issues relating data obtained in an experimental setting to that which occurs in people. Recent examples of these types of studies include the link between aflatoxin exposure and liver cancer and exposure to benzene and a number of adverse human health outcomes. As this research continues, there will be an ever increasing push to translate experimental findings for other carcinogens to population-based investigations. The combination of mechanistic studies, epidemiology and risk assessment offers great potential to understand the potential for health impact from exposures to environmental agents. Thus, these studies are central to modern risk assessment technologies. Finally, these studies are being used to develop clinical intervention protocols for high risk individuals.

908 GENETIC TARGETS OF ENVIRONMENTAL CARCINOGENS

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Animal studies are elucidating relations between carcinogen exposure dose, total and individual DNA adducts in target organs, and final tumor response. Such quantitative molecular dosimetry experiments in rainbow trout have shown a predictive and direct relationship between the ability of certain anticarcinogens to reduce initial DNA damage and their influence on carcinogen dose-response TD_{50} values. By comparison, the specific gene targets crucial to tumor initiation and progression, and the levels and preferred sites of damage sustained in these genes, are less well defined. Mutations in *Ras* proto-oncogenes and p53 tumor suppressor genes are likely candidates for damage by environmental carcinogens. Experimental tumors frequently exhibit codon 12, 13, or 62 mutations in *Ha-*, *Ki-*, or *N-ras* genes. Mutations can vary in frequency and identity according to carcinogen, animal, target organ, and protocol used. Thus 7,12-dimethylbenzanthracene gives different incidences and types of *Ras* mutations in mouse skin, mouse liver, and trout liver tumors. A similar observation holds for N-nitrosodiethylamine and dibenzo(a,l)pyrene, presumably due to model differences in metabolism, DNA repair, and perhaps differential *Ras* allele selectivity. This variability may preclude the use of animal mutational data by molecular epidemiologists to identify sources of human cancer. The UV-related p53 mutations in human skin cancer are an exception. Finally, there is concern that artificial animal

protocols may not accurately invoke molecular pathways common to human cancer development. Thus, AFB_1-like p53 codon 249 G-T transversion frequently observed in AFB_1-exposed hepatocellular carcinoma patients in China has yet to be observed in experimental AFB_1 carcinogenesis, whereas the G-T *Ras* mutations frequently seen in AFB_1-initiated rat and trout liver tumors have not been reported in the humans. Absence of hepatitis-related chronic inflammation in these models may underlie this discrepancy.

909 POLYMORPHISMS IN CARCINOGEN METABOLISM AND HUMAN CANCER SUSCEPTIBILITY

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The bioactivation and detoxification pathways associated with the carcinogenic aromatic amines (AAs) and polycyclic aromatic hydrocarbons (PAHs) provide an excellent model of metabolic polymorphisms that appear to modulate human carcinogenesis. The wide variations in the biotransformation of these agents, whether genetically determined or environmentally provoked, can thus be a significant factor in individual cancer susceptibility. Our current studies are concerned with human cancers of the urinary bladder, colon and rectum, pancreas, and larynx, and the role of enzymatic systems that are known to play a major role in the biotransformation of carcinogenic AAs and PAHs. These include: N-acetyltransferases (NATs); sulfotransferases (STs); glutathione S-transferases (GSTs), and the cytochromes P450 (CYP1A1, CYP1A2, CYP2C9/10). The influence of metabolic polymorphisms in these enzymatic systems on the levels of DNA adducts in human carcinogen-target tissues is being examined as biomarkers of individual susceptibility and of carcinogen exposure.

910 MECHANISM-BASED BIOMARKERS OF AFLATOXIN EXPOSURE IN THE MOLECULAR EPIDEMIOLOGY OF LIVER CANCER

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The incorporation of molecular biomarkers as adjuncts to epidemiologic methods has been particularly effective in the evaluation of aflatoxin exposure in the etiology of hepatocellular carcinoma. For these studies, we developed and validated assays for the aflatoxin-albumin adduct and for aflatoxin metabolites in urine, including one to detect and quantify the major adduct formed between aflatoxin B_1 and guanine. We demonstrated the prevalence of these biomarkers in populations and through a prospective study in Shanghai, PRC, that aflatoxin urinary biomarkers strongly predicted risk of hepatocellular carcinoma independent of hepatitis B and other hepatitis serologies, whereas estimated aflatoxin exposure from dietary survey did not. We demonstrated a strong interaction between hepatitis B surface antigen positivity and the aflatoxin biomarkers with risk of developing hepatocellular carcinoma. We have also developed a new analytical method for carcinogen-DNA adduct measurement to provide reliable quantitative data. The procedure involves adduct detection by acylation with methionine (ADAM). In the experimental strategy used, mononu-cleoside adducts are subjected to acylation with methionine through the use of a reagent (t-butoxycarbonyl-L-[^{35}S]methionine, N-hydroxysuccinimidyl ester), followed by separation by HPLC of the derivatized products. ADAM, unlike ^{35}P-postlabeling, is based on modification of adducts in the nucleoside form, a procedure that is applicable for all chemical classes of adducts since it depends only on the availability of free hydroxyl (and/or amine) groups. Results to date have demonstrated the utility of this approach for detection and analysis of structurally diverse carcinogen adducts isolated from digests of cellular DNA or urinary adducts produced through excision repair mechanisms.

911 MOLECULAR CYTOGENETICS OF HUMANS EXPOSED TO BENZENE

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Fluorescence *in situ* hybridization (FISH) is a powerful new tool for investigating mechanistic events in human cancer epidemiology. It allows numerical chromosomal aberrations (aneuploidy) to be detected in interphase and structural changes to be analyzed in metaphase spreads by chromosome painting. We have used FISH to perform cytogenetic analyses in the blood cells of workers exposed to benzene. Forty-three workers exposed to benzene (median = 31 ppm) and 44 age- and sex-matched controls were studied in Shanghai, China. High benzene exposures increased the hyperdiploid frequencies of chromosomes 7, 8 and 9. Only chromosome 7 was significantly affected at exposure levels below 31 ppm. There was, however, a significant correlation between hyperdiploidy of all three chromosomes and benzene exposure. Hypodiploidy also occurred more frequently in the exposed workers. The effect of benzene on chromosomes 7 and 8 is intriguing because monosomy 7 and trisomy 8 are common cytogenetic abnormalities in leukemia. Another common cytogenetic abnormality in leukemia is the t(8;21) translocation. We painted chromosomes 8 and 21 different colors on metaphase spreads allowing for easy detection of t(8;21). More than 77,000 cells were scored, which would not have been feasible by conventional cytogenetics. Benzene exposure produced a dose-dependent increase in t(8;21) translocations that was highly significant. Our data therefore shows that benzene can produce aneuploidy of chromosomes 7 and 8 and the t(8;21) translocation in exposed humans. These events may be important in the development of benzene-induced leukemia. This work was supported in part by NIH grant RO1 ES 06721.

912 MODULATION OF T-HELPER CELL POPULATIONS; POTENTIAL MECHANISMS OF RESPIRATORY HYPER-SENSITIVITY & IMMUNE SUPPRESSION: INTRODUCTION

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There are two major subdivisions of $CD4^+$ helper T lymphocytes which regulate different sets of immune responses. Th1 cells produce interleukin (IL) 2 and γinterferon as well as other cytokines, mediate delayed-type hypersensitivity responses, provide help for complement-fixing antibodies, activate macrophages, and may be particularly important in responding to viral and tumor antigens expressed on cell surfaces. Th2 cells produce a different array of cytokines, including IL-4 and IL-5, which promote IgG1, IgA, and IgE responses, and enhance eosinophil differentiation. Th2 cells are more important in responding to parasitic infections and the generation of immediate-type hypersensitivity responses, including reactions to allergens and some types of asthma. The balance between Th1 and Th2 cells is affected by infections, aging, hormones, and toxic substances. Such alterations may impact the outcome of infectious, neoplastic, allergic, and autoimmune diseases. It is apparent that one way in which chemicals might produce immunotoxic effects is via modulation of T helper cell populations. This symposium presents examples of several agents which appear to act in this manner and describes both underlying mechanisms & impacts on susceptibility to and expression of infectious & allergic disease. This abstract does not reflect EPA policy.

913 REGULATORY T CELLS AND THEIR MODULATION BY METALS

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Evidence to address the mechanisms(s) by which lead and mercury suppress host resistance to numerous infectious agents and cancers (inhibition of cell-mediated immunity) but initiate or enhance humoral immune responses to self antigens will be presented. These metalloregulatory effects are suggested to result from differential activation of the $CD4^-$ helper T-lymphocyte subsets (Th2 and Th2) that produce IL-2 and IFNγ and IL-4, IL-5, IL-6 and IL-10, respectively. Lead and mercury appear to possess some of the characteristics of superantigens in that they stimulate non-MHC restricted T cell proliferation and block antigen-specific T cell activation. Like superantigens, they also skew the antigen specificity of the T cells' receptors (TCR) by enhancing and inhibiting the expression of select Vβs. The differential activation of the Th1 and Th2 subsets involves a combination of changes between the costimulatory molecules (e.g., CD80 and CD86) on antigen-presenting cells, production of regulatory cytokines (mainly IL-4, IL-10, IL-12, and IFNγ), and signal transduction differences betwween Th1 and Th2 cells. The *in vitro ex vivo* and *in vivo* influence of metals on the development of Th1 and Th2 cells from the Thp (precursor) population will be discussed as well as the ability of lead and mercury to directly modify the activities of the Th1 and Th2 cells and other immunogregulatory cells. The importance of the Th1:Th2 ratio in maintenance of health also will be discussed.

914 INDUCTION OF A TH-2 LIKE IMMUNE REACTION FOLLOWING EXPOSURE TO UV RADIATION

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In addition, to being the primary cause of skin cancer, UV radiation is immune suppressive and there appears to be a link between the ability of UV to suppress the immune response and induce skin cancer. Cytokines made by UV-irradiated keratinocytes play an essential role in activating immune suppression. In particular, we have found that keratinocyte-derived IL-10 is responsible for the systemic impairment of antigen-presenting cell function and the UV-induced suppression of delayed-type hypersensitivity (DTH). Antigen presentation by splenic adherent cells isolated from UV-irradiated mice to T helper 1 type T (Th1) cells is suppressed, whereas, antigen presentation to T helper 2 type T (Th2) cells is enhanced. The enhanced antigen presentation to Th2 cells, and the impaired presentation to Th1 cells can be reversed in vivo by injecting the UV-irradiated mice with monoclonal anti-IL-10 antibody. Furthermore, immune suppression can be transferred from UV-irradiated mice to normal recipients by adoptive transfer of T cells. Injecting the recipient mice with anti-IL-4 or anti-IL-10 prevents the transfer of immune suppression, suggesting the suppressor cells are Th2 cells. In addition, injecting UV-irradiated mice with IL-12, a cytokine that has been shown to prevent the differentiation of Th2 cells in vivo, reverses UV-induced immune suppression. These findings support the hypothesis that UV exposure activates IL-10 secretion, which depresses the function of Th1 cells, while enhancing the activity of Th2 cells.

915 REGULATION OF IMMUNE RESPONSES TO HOUSE DUST MITE ANTIGEN IN ALLERGIC LUNG DISEASE

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Allergic asthmatics exhibit a different immune phenotype than normals and experience damaging inflammatory and physiologic responses after inhalation of specific allergens. It is now known that immune responses occuring in the body are governed by two different phenotypes of T lymphocytes (Th1 and Th2) which are mutually antagonistic to each other by virtue of their cytokine profiles. Allergic asthmatics display a Th2 immune phenotype which is known to promote IgE antibody production and recruitment of eosinophils. Exactly how these responses are initiated in these individuals is unclear although it is known that allergy can be hereditary, and that exposure to antigen at critical periods of immune development is important. Also, infections and co-exposure to other xenobiotic agents such as air pollutants may exacerbate particular aspects of pulmonary immune-mediated disease by augmenting inflammatory and immunostimulatory mechanisms. This presentation will focus on a model of allergic lung disease in rats sensitized to house dust mite, the mechanisms involved in producing immune-mediated inflammation and airway hyperreactivity as well as examples of how air pollutant exposure can modulate immune function in favor of Th2 responses and subsequent clinical disease. Further examples will illustrate basic mechanisms for the elicitation of different immune responses and how cytokine therapy can modualte immune function to a less harmful phenotype. This abstract does not reflect EPA policy.

916 DYSFUNCTION OF PULMONARY IMMUNITY IN ATOPIC ASTHMA: POSSIBLE ROLE FOR T HELPER CELLS

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Atopic asthma is characterized by Th2 type immune response to antigens to which sensitization has previously occurred. To determine if this tendency extends to antigens not previously encountered, we examined the systemic and pulmonary response to pulmonary immunization with a neoantigen. We instilled 500 μg keyhole limpet hemocyanin (KLH), a well described T dependent antigen, into a subsegment of the left lung of 9 atopic asthmatics and 9 non atopic controls and examined serum anti-KLH IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, IgA_2 and IgM and peripheral blood mononuclear cell anti-KLH antibody production for 25 days thereafter. We also determined specific antibody in bronchoalveolar lavage fluid (BALF) in both the immunized and a control lobe 11 days after immunization.

We found specific serum antibody in all immunized subjects with no difference between atopics and normals in the amount or kinetics of anti-KLH IgG_1, IgG_2, IgG_3, IgA_1, IgA_2 and IgM. However, the atopics exhibited more anti-KLH IgG_4 than the normal controls ($p<.05$). Specific anti-KLH antibody producing cells were detected in peripheral blood in most subjects at day 8 to 12 after immunization with no difference between atopics and normals. Specific IgA1 and IgA2 antibody was detected in BALF from the immunized lobes of both groups of subjects with no difference between atopics and normals.

We conclude that atopic asthmatics respond to intrapulmonary KLH with more serum anti-KLH IgG_4 than normal controls, consistent with a bias towards a Th2 response to intrapulmonary exposure to antigen.

917 FUNCTIONAL MATURATION OF Th CELL RESPONSES AND DIFFERENTIAL CYTOKINE PRODUCTION FOLLOWING CHEMICAL SENSITIZATION

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Many chemicals are able to induce allergic contact dermatitis, whereas others are associated primarily with sensitization of the respiratory tract. The characteristics of immune responses induced in mice by contact and respiratory chemical allergens has been investigated with respect to the development of differentiated T helper (Th) cell function. Following acute primary exposure of mice to either class of allergen activated draining lymph node cells have been shown display increased expression of both the Th1 cell cytokine interferon γ (IFN-γ) and the Th2 cytokines interleukins 4 and 10 (IL-4 and IL-10). Such is consistent with the stimulation early during immune responses of Th0 cells that elaborate both Th1- and Th2-type cytokines. Following more chronic exposure to chemical allergens, however, phenotypes of selective cytokine secretion develop. Activated lymph node cells isolated from mice treated with chemicals known to cause sensitization of the respiratory tract produce substantial amounts of IL-4 and IL-10, but little IFN-γ, indicative of preferential Th2 cell activation. Conversely, contact allergens considered not to induce respiratory sensitization provoke vigorous IFN-γ secretion, but only comparatively low levels of IL-4 and IL-10; a pattern compatible with the selective stimulation of Th1 responses. The evolution of divergent immune responses determines the characteristics of allergic reactions and provides a molecular basis for the identification and classification of chemical sensitizers.

918 LIVER CONCENTRATIONS OF HEPTA-CDD IN A 26-WEEK STUDY

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Groups of 20 male and 20 female rats were given five different doses (males: 30 to 10,000 μg/kg; females: 18.5 to 6,000 μg/kg) of 1,2,3,4,5,6,7,8-heptachlorodibenzo-*p*-dioxin (hepta-CDD) or one dose (males: 70 μg/kg; females: 41.9 μg/kg) of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (tetra-CDD) divided into four daily loading doses and six biweekly maintenance doses. The dosing period was 13 weeks after which half of the rats were necropsied and the rest allowed to recover for a period of another 13 weeks. About 0.2 g of each lyophilized rat liver was extracted, the extract purified by column chromatography and analyzed by GC/MS for CDD content. Liver concentration of hepta-CDD reflected quite accurately the dosage ratios in both males and females for the entire dose range indicating that the toxicokinetics of hepta-CDD is independent of dose in a more than 300-fold dosage range. Liver concentrations of hepta-CDD decreased between the end of dosing (90 days) and termination of the experiment (180 days) by 19.3 ± 9.2 and 17.3 ± 3.2% for males and females, respectively. This indicates a liver half-life for hepta-CDD of 237 days in males and 260 days in females. The much longer half-life of hepta-CDD in a subchronic/chronic study than after acute exposure is due to a greatly reduced growth dilution. Liver concentration of tetra-CDD decreased during the same period of time by 99.4 and 98.1% for males and females indicating respective half-lives of about 13 and 15 days. The fact that tetra-CDD has a much higher binding affinity for certain liver proteins than does hepta-CDD, in combination with above findings indicates that it is not protein binding but most likely lipid solubility that is the key factor that drives the toxicokinetics of CDDs.

919 DISPOSITION OF PCBs IN B6C3F1 MICE FOLLOWING 13 WEEKS OF EXPOSURE

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PCBs are found in the environment as components of complex mixtures. A subset of the PCBs are ligands for the Ah receptor and produce similar toxicities as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The present study examines the SAR for disposition of several dioxin-like PCBs. Three coplanar PCBs, [3,3',4,4'-tetrachlorobiphenyl (77); 3,3',4,4',5-pentachlorobiphenyl (126); 3,3',4,4',5,5'-hexachlorobiphenyl(169)], 3 mono-ortho-substituted PCBs [2,3,3',4,4'-pentachlorobiphenyl (105); 2,3',4,4',5-pentachlorobiphenyl (118); 2,3,3',4,4',5-hexachlorobiphenyl (156)] and TCDD were examined. Chemicals were administered to female B6C3F1 mice by gavage in corn oil, 5d/wk for 13 wks. Three days after the last dose, the study was terminated and chemical levels were determined in liver, fat, skin and blood. Dose-dependent hepatic sequestration as determined by dose-dependent increases in both %dose/g liver and liver/fat ratios were observed for TCDD, consistent with previously published reports. Dose dependent increases in both % dose/g liver and liver/fat ratios were observed for PCBs 126 and 169. Concentrations of TCDD in liver ranged from 2 – 6%. Hepatic concentrations of PCBs 126 and 169 ranged from 0.01–0.1 % dose/g liver while all other PCBs were less than 0.001 % dose/g liver. Liver/fat ratios were greater than 1 for TCDD and 126, indicating hepatic sequestration. When liver/fat ratios were normalized for acetanilide-4-hydroxylase activity, a marker for CYP1A2, PCB 126 had a greater affinity for hepatic tissue than TCDD. All other PCBs had liver/fat ratios less than 1. Dose-dependent decreases in % dose/g fat were observed for TCDD and 126, while dose-dependent increases in % dose/g fat were observed for 118. Dose-dependent decreases in % dose/g skin were observed for TCDD, 126, and 118. Of the PCBs examined only 126 and 169 had dose-dependent dispositions similar to TCDD. The remaining PCBs had greater affinity for adipose tissue than hepatic tissue. (This abstract does not necessarily represent EPA policy).

920 TCDD INDUCES CATECHOL ESTROGEN METABOLISM AND CYP1B1 IN ACHN HUMAN RENAL ADENOCARCINOMA CELLS

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent inducer of cytochromes P450 of the *CYP1* gene family. The three known human enzymes of the *CYP1* family, CYP1A1, CYP1A2 and CYP1B1, all appear to be 17β-estradiol (E_2) hydroxylases. In this study the ACHN cell line, derived from a human renal adenocarcinoma, was used as a model system to investigate the effects of exposure to TCDD on E_2 metabolism in human renal tissue. Exposure of ACHN cultures to 10 nM TCDD caused a greater than 10-fold increase over control in the rate of microsomal E_2 metabolism. The C-4 position of E_2 was the primary site of hydroxylation catalyzed by microsomes from TCDD-treated ACHN cells; the rate of E_2 2-hydroxylation was approximately half the rate of 4-hydroxylation. Stimulation of the E_2 4-hydroxylation pathway by exposure to TCDD was also evidenced in cellular metabolism experiments, as 4-methoxyestradiol was the principal metabolite of E_2 released into the medium of TCDD-treated cultures. Northern blot analyses showed a 20-fold induction of the 5.1 kb CYP1B1 mRNA in ACHN cells in response to treatment with TCDD, while the 2.6 kb CYP1A1 mRNA was only marginally induced. Thus, TCDD-treated ACHN cells provide a unique opportunity to investigate CYP1B1 expression with minimal interference from CYP1A1. They also provide an *in vitro* system to study the E_2 4-hydroxylation pathway, which is associated with renal carcinogenesis in experimental animals. Supported by grants 2P42-ESO4913, ESO6071 and ESO7141.

921 EVIDENCE FOR THE INDUCTION OF APOPTOSIS BY 2,3,7,8 TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN VIVO

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TCDD is a highly toxic environmental contaminant. One of the characteristic features of TCDD-induced toxicity is its action on the thymus. The exact mechanism by which TCDD induces thymic involution is not clear. In the current study we investigated whether TCDD triggers apoptosis of thymocytes when administered *in vivo*. Administration of a single dose of TCDD (50μg/kg) into C57BL/6 strain intraperitoneal caused significant but delayed thymic atrophy ~5 days after the injection. When thymic apoptosis was studied using fluorescein dUTP and the cells were analyzed using a flow cytometer, significant apoptosis in thymocytes was detected ~12 hours after TCDD injection. Interestingly, no significant apoptosis was seen beyond 1–5 days after TCDD administration. When this effect was compared to dexamethasone administration *in vivo* (100mg/kg), it was noted that dexamethasone caused dramatic decrease in thymic cellularity within 24 hours. Also, with dexamethasone, marked apoptosis was detected at ~12 hours after the drug administration while apoptosis was not detected at subsequent days post-administration. Our data suggest that TCDD-induced thymic atrophy is a slow process involving apoptosis and therefore the apoptotic cells may be cleared by the phagocytic cells *in vivo* thereby making it difficult to demonstrate apoptosis at later stages. We have also observed that Fas^+ (C57BL/6+/+) mice are more sensitive to TCDD-mediated thymic atrophy as well as peripheral T cell dysfunction when compared to Fas^- (C57BL/6 lpr/lpr) mice. Together our data suggest that apoptosis may play a crucial role in TCDD-mediated immunotoxicity.

922 SYNERGISTIC HEPATIC PORPHYRIN ACCUMULATION AND URINARY PORPHYRIN EXCRETION AFTER SUBCHRONIC EXPOSURE TO MIXTURES OF PCDDS, PCDFS, AND PCBS IN MICE

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Subchronic studies involving exposure to mixtures of polychlorinated dibenzodioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) (planar and mono-ortho substituted PCBs) resulted in additive effects on induction of CYP1A1 and CYP1A2 activities (Hurst *et al.* (1995); The Toxicologist, 15, 63). Here we report on hepatic porphyrin accumulation and urinary porphyrin excretion in female B6C3F1 mice after 13-week (5 days/wk) oral exposure to a mixture of 1:PCDDs, PCDFs, and PCBs (only planar and mono-ortho substituted PCBs) (0.45, 1.5, 15, and 150 ng TEQ/kg/day); 2: same mixture as 1 with the addition of the di-ortho substituted PCB 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) (0.45, 1.5, 15, and 150 ng TEQ/kg/day with 7.0, 23, 232, and 2323 μg PCB 153/kg/day; 3: PCB 153 alone (7.0, 23, 232, and 2323 μg PCB 153/kg/day); 4: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) alone (0.45, 1.5, 15, and 150 ng TCDD/kg/day). Only the highest dose groups (with the exception of PCB 153) resulted in hepatic porphyrin accumulation and urinary porphyrin excretion. TCDD (150 ng/kg/day) resulted in 26 μg porphyrins/g liver, which was 50 times control levels. The PCDD, PCDF, and PCB mixture (without PCB 153) gave 2101 μg porphyrins/g liver (4000 times control levels). The mixture with PCB 153 resulted in 3420 μg/g liver (6800 times control levels). No effect was found for PCB 153 alone at any dose. Urinary porphyrin excretion was first detected after 6 weeks of exposure to TCDD and both mixtures which correlated with hepatic porphyrin accumulation. These results indicate strong synergistic effects on porphyrin metabolism after exposure to mixtures of PCDDs, PCDFs, and PCBs in comparison to TCDD alone.
[This abstract does not necessarily represent EPA policy].

923 CORRELATION BETWEEN UROPORPHYRIA AND HEPATIC TOXICITY INDUCED BY THE SYNERGISTIC INTERACTION OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) AND IRON IN Ah^{b-1}, Ah^{b-2} AND Ah^d MICE

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Previous evidence suggests that uroporphyria induced by TCDD in mice is potentiated by iron and may not depend absolutely on the Ah-genotype. Whether such an effect on more general aspects of liver toxicity occurs has not been clearly established, although it has been suggested some years ago. This may have important implications for the mechanism of hepatic carcinogenicity. Male C57BL/6J, BALB/c, SWR and DBA/2 mice received dextran or iron-dextran (600 mg Fe/kg) and then after one week either oil or TCDD (75 μg/kg). Five weeks later mice were culled. Iron potentiated porphyria induced by TCDD not only in C57BL/6J mice but also in those of the BALB/c and SWR strains. Similarly, serum ALT levels were increased by dual treatment in all 3 strains. Histologically, iron clearly potentiated liver damage caused by TCDD in each of these strains. The greatest responses

were always in Ah^{b-1} C57BL/6J mice and less, but of a similar nature, in Ah^{b-2} BALB/c and Ah^{d} SWR mice. In contrast Ah^{d} DBA/2 mice given this dose were resistant to all toxic effects. Thus the hepatic toxicity as well as porphyrogenic activity of TCDD is influenced by genes in addition to Ah alleles, and may involve iron-catalysed free radical mechanisms.

924 HISTOMORPHOLOGIC EVALUATION OF A 26-WEEK STUDY WITH HEPTA-CDD

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Groups of 20 male and 20 female rats were given five different doses of 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (hepta-CDD). The doses were selected based on the relative potency factors derived from acute toxicity studies and toxicokinetic considerations. Total doses for males and females (30.9/18.5, 370/222, 2222/1333, 6667/4000 and 10,000/6000 μg/kg) were divided into four daily loading doses and six biweekly maintenance doses. Positive controls were administered 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (tetra-CDD; 70/41.9 μg/kg). The dosing period was 13 weeks after which half of the rats were necropsied and the rest allowed to recover for another 13 weeks. A state-of-the-art histomorphologic examination implicated by Fisher's Exact Test (one-tailed, $p < 0.05$) the following deviations from normal morphology: hepta-CDD, 90 days, males: stomach, mesenteric lymph node, spleen, thymus, lungs, liver, kidney (right) testis and teeth; females: stomach, ileum, thymus, liver, mammary gland, teeth; tetra-CDD, 90 days, males: stomach, mesenteric lymph nodes, spleen, thymus, lungs, liver, testis and teeth, females: ileum, thymus, liver and teeth. There was virtually no recovery observable in hepta-CDD-treated rat after the 90 day off dose period but additional tissues/organs were also implicated such as the thyroid, brown adipose tissue and the pituitary. Some reversibility occurred in tetra-CDD-treated rats. Most findings are in agreement with differential toxicokinetics of these two CDDs in the rat (see Rozman *et al.*, ibid). They also support the notion that the AUC (area under the curve; dose x time = constant) is the primary determinant of subchronic/chronic toxicity of CDDs.

925 ACUTE 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN DOSING ALTERS HEPATIC EXPRESSION OF CELL CYCLE CONTROL PROTEINS - EVIDENCE FOR CELL CYCLE ARREST *IN VIVO*

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The cell cycle is a comprised of complex signaling pathways that is coordinated by an orchestra of cellular players. These include the positive effectors (early response genes), cyclin dependent kinases, (CDKs), cyclin proteins, and negative regulators (tumor suppressor proteins and CDK inhibitory proteins). Previous work in our laboratories has demonstrated that acute 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) dosing alters homeostatic expression of cell division control proteins cyclin dependent kinase 1 (CDK1), p53, and p21*Waf1*. In this study, we have further examined TCDD's effects on the expression of other related cell cycle control proteins that function in the liver of Balb/c mice. Female Balb/c mice were administered a single i.p. dose of TCDD (0.25, 0.5, 1, or 2 μg/kg) or an equal volume of the corn oil carrier. Six days post-dosing the animals were sacrificed, livers were removed, homogenized, centrifuged at 9000 x g, and the supernatant (S9) fractions were collected. The S9 proteins were prepared for SDS-PAGE and western blot analysis. Subsequent probing for cell cycle proteins demonstated that TCDD produced elevations in CDK2, CDK4, CDK5, cyclin D2 and p16, a CDK inhibitory protein and proposed tumor suppressor protein. These results provide further evidence that TCDD induces a proliferative signal within hepatocytes that can possibly be attenuated by tumor suppressor proteins yielding hepatic cell cycle arrest *in vivo*.

926 HISTOMORPHOLOGIC EVALUATION OF A 26-WEEK STUDY WITH A MIXTURE OF FOUR CDDs

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Groups of 20 male and 20 female rats were given five different doses of a mixture of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (tetra-CDD), 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (penta-CDD), 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (hexa-CDD) and 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (hepta-CDD). The doses were selected based on relative potency factors derived from acute toxicity data and a previous subchronic study with hepta-CDD. The mixture was constituted such that each homologue contributed one fourth to total toxicity. Total doses were divided into four daily loading doses and six biweekly maintenance doses. The highest total dose for males was 17.5 μg/kg of tetra-CDD, 87.5 μg/kg of penta-CDD, 350 μg/kg of hexa-CDD and 2500 μg/kg of hepta-CDD. Positive controls received penta-CDD (350 μg/kg) or hexa-CDD (1400 μg/kg). Females were given 1.5 times lower doses. The dosing period was 13 weeks with mid-term necropsies at 90 days followed by another 13 weeks of recovery for the remaining half of the rats. A state-of-the-art histomorphologic examination implicated by Fishers' Exact Test (one-tailed, $p < 0.05$), the following deviations from normal morphology: mixture, 90 days, males: mesenteric lymph node, spleen, thymus, lungs, liver, kidneys, bone marrow and teeth; females: duodenum, mesenteric lymph node, spleen, thymus, liver, heart, cervical lymph node, bone marrow and teeth. After the recovery period (180 days) a few lesions were reversed while others not present at 90 days appeared. The two positive control groups showed great similarity to each other and to the mixture groups in terms of lesion patterns. Visual inspection of the data indicated though a slight congener specificity.

927 SUBCELLULAR DISPOSITION OF [^{3}H]2, 3, 7, 8 - TETRACHLORODI-BENZO-*P*-DIOXIN (TCDD) IN THE LIVER AND KIDNEYS OF FEMALE SPRAGUE-DAWLEY RATS AFTER ACUTE EXPOSURE: ROLE OF CYP1A2

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We investigated the role of CYP1A2 in subcellular localization of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the liver and kidneys of female Sprague Dawley rats acutely exposed (3 days) to 0.01, 0.1, 0.3, 1.0, 10.0 and 30.0 μg [^{3}H]TCDD/kg. Highest concentrations of [^{3}H]TCDD are observed within the liver with a dose-dependent decrease in [^{3}H]TCDD levels occurring in the kidneys. Analysis of the subcellular fractions from TCDD-treated rats indicates that [^{3}H]TCDD localizes to the P9 (mitochondrial, lysosomal, and nuclear) fraction in all tissues. Separation of the S9 (cytosol and microsomal) fraction into the P100 (microsomal) and S100 (cytosolic) fractions suggests a tissue-specific localization of [^{3}H]TCDD. Most of the [^{3}H]TCDD within the hepatic S9 fraction localized to the hepatic P100 fraction; however, [^{3}]TCDD levels within the renal S9 fractions localized to the S100 fraction. Western blot analysis of S100 fractions containing the aryl hydrocarbon (Ah) receptor (AhR) and AhR nuclear translocator (Arnt) proteins revealed that expression of these proteins was dose-independent. Analysis of the functionality associated with TCDD-exposure revealed a dose-dependent increase in the induction of CYP1A1 protein and associated enzyme activity in the hepatic and renal microsomes. TCDD-induced CYP1A2 protein levels and associated enzymatic activity was localized to the hepatic microsomes. This data supports the hypothesis that the hepatic sequestration of TCDD is due to interaction of the ligand with the hepatic ligand-inducible microsomal binding protein, CYP1A2. (Supported by USEPA Cooperative Training Agreement (T-901915-02) with UNC-CH). (This abstract does not necessarily represent USEPA policy).

928 IMPACT OF MUTATED c-Ha-*ras* TRANSFECTION AND EXPRESSION OF ARYL HYDROCARBON RECEPTOR (AhR) FUNCTION

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MCF10A is a spontaneously immortalized human epithelial cell line that has the phenotypic and genotypic characteristics of normal breast cells. Exposure of MCF10A cultures to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) resulted in the transcriptional activation of the *CYP1A1, CYP1B1* and *NMO1* genes. Induction of these genes is mediated by the interaction of TCDD with the aryl hydrocarbon receptor (AhR). Northern blot and nuclear run-on assays demonstrated that transcriptional activation of these genes was suppressed in stably transfected cultures expressing a mutated c-Ha-*ras* oncogene, and constitutively activated p21-ras protein (MCF10A-NeoT line). Similar suppressions did not occur in stably transfected lines carrying the expression vector (MCF10A-Neo) or a normal c-Ha-*ras* gene (MCF10A-neoN). Western blot analyses and immunofluorescence microscopy demonstrated that the lack of inducibility in MCF10A-NeoT cells reflected neither reductions in AhR and ARNT contents, nor impediment of TCDD-induced AhR translocation to the nucleus. Suppression instead correlated with reductions in DNA-AhR complex formation, as analyzed in gel retardation assays of cytoplasmic extracts treated *in vitro* with TCDD. These studies suggest that the p21-ras protein controls signal transduction pathways capable of modulating AhR function. (Supported by NIH grant CA34469).

929 ARE CYP1A1 AND UGT1*6 CO-INDUCED IN HUMAN LUNGS?

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CYP1A1 has been demonstrated in the lungs of smokers. Tobacco smoke is a source of a whole array of carcinogenic chemicals, including carcinogenic polynuclear aromatic compounds. About 10% of the humans may exhibit high CYP1A1 inducibility; these people may thus have greater risk than individuals with low inducibility for cigarette smoke-caused cancer. The induction of one of the UDPglucuronosyl-transferases, UGT1*6, has been proposed to be regulated in mouse liver by the ligand-activated transcription factor (AhR) similarly to CYP1A1. The aim of this study is to examine the expression of CYP1A1 and UGT1*6 in human lungs in smokers and non-smokers.

The CYP1A1 mediated hydroxylation of pyrene and the expression of CYP1A1 was analysed in human lungs, together with the UGT1*6 activity toward 1-hydroxypyrene and its expression by Western blotting. The pulmonary pyrene-1-hydroxylase activity was about ten times higher in smokers (6.1 ± 0.7 pmol/min/mg, n = 53) than in current non-smokers (0.7 ± 0.1 pmol/min/mg, n = 48). The oxidation product, 1-hydroxypyrene, is a good substrate for UDPglucuronosyltransferases. The activity in human lung microsomes ranged from 0.3 to 216 pmol/min/mg protein. There was no significant difference between smokers and non-smokers (3.8 ± 0.5 pmol/min/mg vs. 8.4 ± 4.5 pmol/min/mg). The 1-hydroxypyrene glucuronidation rates or UGT1*6 protein amounts did not correlate with 1-hydroxypyrene formation, suggesting that only CYP1A1 induction is strongly related to smoking but not the activity or amount of UGT1*6 in human lungs.

930 EXPRESSION OF CYTOCHROME P450 (P450) IN BREAST TUMORS AND NORMAL TISSUES DETECTED BY RNA-PCR

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P450s in brest tissue can play a role in breast carcinogenesis by metabolically bioactivating xenobiotics and endogenous componds such as estrogen, and altering the efficacy of therapeutic drugs. However, very little is known of the P450 composition expressed in the human breast. We have employed a sensitive and specific method, RNA-polymerase chain reaction (RNC-PCR) followed by probing with squence specific digoxigenin-labelled probes (either oligonucleotide or DNA), to study the profile of P450s in a panel of normal breast tissues and breast tumors. DNA impurities in extracted RNA samples were removed by DNase I treatment. Normal tissue was obtained from reduction mammoplasty (6) and from regions surrounding tumors (5). We detected CYP1A1 mRNA in 55% (6/11) of normal tissues and 23% (3/13) of tumors, CYP3A4 in 73% (8/11) of normal tissues and 31% (4/13) of tumors, and CYP2D6 in 91% (10/11) of normal tissues and all (13/13) tumors. No CYP mRNAs were detected in tissues from 2 patients with fibroadenoma. We also detected strong expression of DT-diaphorase mRNA, a phase II enzyme, in all normal and tumor tissues. Other forms of P450 mRNA are under study. These results will provide insights into the potential of xenobiotics, including therapeutic drugs, and endogenous compounds to be metabolized in breast tissue.

931 HUMAN CYTOCHROME P450 (CYP) 1B1 IS A LOW Km 17β-ESTRADIOL (E_2) 4-HYDROXYLASE

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Members of the CYP1 family, CYP1A1 and CYP1A2, catalyze the 2-hydroxylation of E_2. In cells in culture, we have shown that E_2 4-hydroxylation correlates with the expression of an additional CYP1 member, CYP1B1. To determine whether human CYP1B1 is an E_2 4-hydroxylase, the human CYP1B1 protein was expressed under a galactose inducible promoter in *Saccharomyces cerevisiae*, producing 300 pmol of P450 per mg of microsomal protein. Kinetic analyses demonstrated that CYP1B1 catalyzed low Km 4- and 2-hydroxylations of E_2 in a ratio of 3:1. The Km values were 0.85 and 1.10 μM for the 4- and 2-hydroxylase activities, respectively. CYP1 gene transcription increases following activation of the aryl hydrocarbon receptor (AhR) by agonists including dioxins, polycyclic aromatic hydrocarbons, and certain indole carbinol compounds. Levels of CYP1B1 and CYP1A1 RNAs increased in a dose-dependent manner in MCF-7 cells treated with either TCDD or indolo[3,2-b]carbazole (ICZ). The EC_{50} for the induction by TCDD was 0.1 nM for CYP1B1 and 0.4 nM for CYP1A1; ICZ was about 1000 times less potent. Treatment of MCF-7 cells with ICZ resulted in a TCDD-like profile of E_2 products, with increased hydroxylation at the C-2, -4, -6α, and -15α positions. The expression of CYP1B1 in numerous human tissues and the specific physiologic and pathophysiologic roles emerging for the E_2 catechols underscore a unique component of the effects of AhR agonists that should be considered in breast cancer intervention studies that are based on this mechanism of enzyme induction. Supported by grants ES06071, ES07141, and 2P42-ES04913.

932 EXPRESSION OF CLASS 3A CYTOCHROME P450 IN MCF10A HUMAN BREAST EPITHELIAL CELLS

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The Class 3A cytochromes P450 (CYP3A), which constitute the major class of cytochrome P450 expressed in human liver, metabolize numerous endogenous substances, procarcinogens and drugs, including agents used in cancer therapy such as tamoxifen and taxol. Although CYP3A expression has been demonstrated in several extrahepatic tissues, little is known about CYP3A expression in hormone responsive tissues that are common sites for human cancer development, such as the breast. We used the reverse transcriptase-polymerase chain reaction (RT-PCR) technique to examine whether any CYP3A genes are expressed in MCF10A, a spontaneously immortalized human breast epithelial cell line that retains many characteristics of normal breast epithelium. Primers corresponding to sequences common to all four known human CYP3A genes were used. A DNA fragment of the predicted size was amplified from total RNA isolated from MCF10A in an amount comparable to that amplified from HepG2, a human hepatoblastoma cell line that is known to express CYP3A7. The PCR product amplified from the MCF10A cells had the same sequence as CYP3A5, a gene that is polymorphically expressed in human liver and intestine. Treatment of MCF10A for 24 hr with either dexamethasone (10^{-5} M) or p,p'-DDT (10^{-5} M) did not increase the amount of CYP3A mRNA detected by RT-PCR, consistent with the previously reported lack of CYP3A5 induction by typical CYP3A inducers. These results demonstrate that MCF10A cells express at least one CYP3A, raising the possibility that CYP3A-catalyzed metabolism in breast could result in local activation of procarcinogens or inactivation of drugs used in the treatment of breast cancer. Supported by NIH Grant HL50710 and by NIEHS Center Grant ES06639.

933 EXPRESSION OF CYP1A1 GENE IN PERIPHERAL LYMPHOCYTES AS A MARKER OF EXPOSURE TO PETROLEUM PRODUCTS IN SERVICE STATIONS

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Several studies have demonstrated a link between the occupational exposure to petroleum products and the elevated risk of acute myeloid leukemia and lung cancer among service station attendants. Because petroleum products contain aromatic compounds such as benzene, an initial interaction of the Ah receptor and induction of phase I and phase II drug-metabolizing enzymes with the petroleum products have been suggested. In our present studies, we have investigated the expression of cytochrome P450–1A1 (CYP1A1) in human blood lymphocytes of petroleum station employees using quantitative competitive Polymerase Chain Reaction (PCR) methodology. 10 ml of venous blood was drawn from 20 males, age 30 to 60, who had been employed by petroleum stations for at least a two year period, and was compared to ten individuals who had not worked in a similiar workplace. The peripheral blood lymphocyte (PBL) was separated on Ficoll Hypaque, and the total RNA was extracted. For each lymphocyte RNA sample, six equal aliquots were prepared. Reverse transcription (RT) of RNA was performed in 20 ul; to these complimentary DNA (CDNA) samples, a dilution series of the internal control was added and PCR was performed in 50 ul. The amplified products were analyzed by electrophoresis and linear regression analysis was used to define the equation for the line through the data point. The results of quantitative competitive RT-PCR clearly indicated 10 to 15 fold more induction of CYP1A1 mRNA in 60% of the individuals exposed to petroleum as compared to non-exposed (control) individuals. Additionally, the higher mRNA levels, in exposed individuals, were well correlated with the length of the exposure to petroleum. Our studies demonstrate a substantial expression of CYP1A1 in individuals exposed to petroleum, and it illustrates a methodology for measuring chemical exposure in humans.

934 INDOLE-3-CARBINOL AND DIINDOLYLMETHANE AS ARYL HYDROCARBON (Ah) RECEPTOR AGONISTS AND ANTAGONISTS IN T47D HUMAN BREAST CANCER CELLS

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Indole-3-carbinol (I3C) is a major component of Brassica vegetables and diindolylmethane (DIM) is an acid-catalyzed dimeric condensation product derived from I3C. Both compounds competitively bind to the aryl hydrocarbon (Ah) receptor with relatively low affinity. In Ah-responsive T47D human breast cancer cells, I3C and DIM did not significantly induce CYP1A1-dependent ethoxyresorufin *O*-deethylase (EROD) activity or CYP1A1 mRNA levels at concentrations as high as 125 or 31 μM, respectively. One nM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induced EROD activity in these cells and cotreatment with TCDD plus different concentrations of I3C (1 to 125 μM) or DIM (1 to 31 μM) resulted in >90% decrease in the induced response at the highest concentration of I3C or DIM. I3C or DIM also partially inhibited (>50%) induction of CYP1A1 mRNA levels by TCDD and reporter gene activity using an Ah-responsive plasmid construct in transient transfection assays. In T47D cells cotreated with 5 nM [^{3}H]TCDD alone or in combination with 250 μM I3C or 31 μM DIM, there was a 37 and 73% decrease, respectively, in formation of the nuclear Ah receptor. The more effective inhibition of induced EROD activity by I3C and DIM was due to *in vitro* inhibition of enzyme activity. Thus, both I3C and DIM are partial Ah receptor antagonists in the T47D human breast cancer cell line.

935 DETECTION OF CYP1A1 IN HUMAN LIVER BY A SPECIFIC MONOCLONAL ANTIBODY

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Questions persist regarding the degree of expression of CYP1A1 in human liver. A specific monoclonal antibody (1–12–3) raised against CYP1A1 from the marine fish scup was shown to cross-react specifically with purified recombinant human CYP1A1, but not recombinant human CYP1A2. Additionally, 1–12–3 detected CYP1A1 in HepG2 cells induced with benzanthracene and analyzed by immunohistochemistry. The use of 1–12–3 with chemiluminescent Western blot detection consistently demonstrated a detection sensitivity as low as 0.01–0.05 pmol CYP1A1 per lane. Utilizing this antibody, the expression of CYP1A1 was quantitated in human liver microsomal samples by either chemiluminescent or colorimetric detection. In contrast to previous studies, all samples tested were positive for the presence of CYP1A1 protein at levels from 0.4 to 5 pmol CYP1A1/mg protein. Ethoxyresorufin-O-deethylase (EROD) activity, a marker for CYP1A enzymes, ranged from undetectable to approximately 120 pmol/min/mg protein. A significant correlation between CYP1A1 content and EROD activity was not observed, possibly due to inactivation in autopsy samples and/or to the EROD activity mediated by the greater levels of CYP1A2 (7–300 pmol/mg). However, a slight increase in EROD activity correlated directly with an increase in CYP1A1 protein in microsomes from human liver slices incubated in the presence of TCDD (0–100 nM). The level of CYP1A2 expression was not increased under these conditions. In conclusion, the monoclonal antibody 1–12–3 is highly specific for human CYP1A1, which was detected in every human liver sample examined, and will prove useful in studies of the cellular localization, induction, and function of CYP1A1 in human tissues. (Supported by NIEHS ES06556 and ES04220)

936 IDENTIFICATION OF TCDD RESPONSIVE CHANGES IN P450 GENE EXPRESSION IN HUMAN LIVER EXPLANTS USING DIFFERENTIAL DISPLAY

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TCDD induces CYP1A and 1B gene expression and alters other gene expression in developing rodent tissues. Previous reports have suggested that human embryonic tissues express functional, xenobiotic-biotransforming P450 isoforms. However, it is not known whether those P450s are subject to TCDD-stimulated gene expression. We recently established an experimental system to induce P450 gene expression in human embryonic tissue explants. The first evidence of explants undergoing induced P450 gene expression was RT-PCR-detection of CYP1A1 mRNA and enhanced biotransformation of ethoxyresorufin. To further elucidate the alteration of gene expression by TCDD in human embryos, it is necessary to isolate genes which are differentially induced by TCDD. Differential Display (DD) is a new approach for identifying genes that may be differentially expressed in 2 or more cell or tissue systems. We also modified this approach by designing a primer with a nucleotide sequence common to most P450 genes in mammals. Human embryonic liver of 45 to 75 days gestation was cultured in Waymouth's medium with or without 1×10^{-7} M TCDD and total RNA was isolated at 6 or 20 hours after the treatment. The total population of mRNAs in the liver explants was subdivided into three groups by first strand synthesis of cDNA with three one-base-anchored oligo-dT primers (Genehunter). The subdivided cDNA population was subjected to PCR using either a combination of the oligo-dT and arbitrary decamers or a 19-mer P450-primer. Our preliminary results indicated 8 bands unique to treated explants and 3 bands unique to untreated explants. Further work is planned to verify these changes in gene expression and characterize the genes expressed. The goal is to identify candidate gene(s) targeted by TCDD in human embryos. Supported by NIEHS grant ES 04041.

937 INDUCTION OF CYTOCHROME P4501A1 (CYP1A1) IN RAT THYMOCYTES AND SPLENOCYTES AND HUMAN LYMPHOCYTES AND NEUTROPHILS FOLLOWING *IN VITRO* EXPOSURE TO TCDD

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CYP1A1 induction is one of the most sensitive responses associated with exposure to TCDD and related compounds and therefore may prove valuable as a biomarker. CYP1A1 mRNA expression and enzyme activity (ethoxyresorufin-o-deethylase, EROD) were investigated in rat thymocytes and splenocytes as well as human peripheral blood lymphocytes and neutrophils. Resting rat thymocytes had detectable levels of EROD activity and CYP1A1 mRNA expression which increased following *in vitro* exposure to TCDD. Conversely, resting rat splenocytes had no detectable EROD activity in the absence or presence of TCDD. Since thymocytes are comprised of many different T lymphocyte subsets, some of which being very immature, possibly one of these subsets is responsible for the resting enzyme activity and is more sensitive to TCDD treatment than mature T lymphocytes. Similar results have been seen after *in vitro* treatment of human lymphocytes and neutrophils with TCDD. However, induction of CYP1A1 enzyme activity or mRNA

levels in human lymphocytes by TCDD was only observed when treated in the presence of concanavalin A (con A), indicating a possible relationship to the cell cycle. Interestingly, the *in vitro* exposure of rat thymocytes to TCDD and con A inhibited the induction of EROD activity. Therefore, biomarker development must consider species-, tissue- and cell cycle-dependent expression of CYP1A1 following exposure to TCDD. (Supported by NIEHS grant ES06556)

938 COMPARING THE REFERENCE DOSE (RfD) AND ESTIMATED SAFE AND ADEQUATE DAILY DIETARY INTAKE (ESADDI) FOR MOLYBDENUM (Mo)

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An ESADDI for Mo, equivalent to a daily dose of 140 μg/day have been established. This estimate is put forth with the proviso that the daily intake of copper (Cu) is in the range of 5–10 mg since Mo is antagonistic to Cu. The ESADDI estimates are made primarily on the basis of animal studies. However, two human nutritional investigations involving Mo deficiency provide supporting data. Abumrad, et al., (1981) provided a report of a patient on long-term parental nutrition that developed amino acid intolerance, irritability and coma. Rajagopalan (1988) reported an inborn error of metabolism. These patients exhibit severe neurologic dysfunction and mental retardation. The USEPA's RfD for Mo of 5 μg/kg-day is based on a Russian epidemiologic study (Koval'skiy, 1961). Elevated serum uric acid is the critical adverse effect reported for 100 adults in a region with high Mo soil content. Seventeen per cent of the affected group had gout-like symptoms. This RfD was assigned an uncertainty factor of 30 and is given medium confidence. The ESADDI estimates are 2.5–4.4 μg/kg-day for infants, 1.9–5.4 μg/kg-day for children, and 1.5–3.6 μg/kg-day for adults. The proximity of the toxicity value, stated in the RfD, to the recommended dietary value for children suggests that toxicologists and nutritionists need to develop compatable methodologies.

939 APPLICATION OF RELATIVE SOURCE CONTRIBUTION (RSC) TO ESSENTIAL TRACE ELEMENTS

D R Borum. *USEPA Washington DC.* Sponsor: K Khanna

When establishing human health criteria for regulatory programs, EPA prepares estimates that reflect human exposure patterns, when data are available. To ensure adequately protective health-based criteria, sources of exposure other than the source for which the particular criterion is being established need to be considered. RSC is the term used to describe the portion of overall exposure contributed by a given exposure source. EPA is currently developing a procedure for all program offices to use when making human exposure evaluations. The approach is proposed for determining RSCs and for subsequently setting health-based criteria. It would also be used for non-regulatory risk characterizations. The proposed procedure, known as the *Exposure Decision Tree*, represents a more comprehensive approach to assessing a chemical, in terms of consideration of the adequacy of available exposure data, relevant sources/media of exposure, quantified levels of exposure, population(s) at risk and relevance to regulatory programs. The *Decision Tree* is proposed for the evaluation of non-carcinogens and other RfD-based chemicals. This procedure is also proposed for essential nutrients. With essential nutrients, consideration must be given to the RDA so that the health-based levels are not too stringent. The implications of setting criteria for essential nutrients will be discussed.

940 MANGANESE: NUTRITIONAL BENEFITS AND ADVERSE EFFECTS MAY OVERLAP IN A HETEROGENOUS POPULATION

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Manganese is a ubiquitous element which is essential for maintenance of health. Frank deficiency of manganese has not been noted because diets typically supply sufficient amounts; however, noted nutritionists have expressed concern that a significant percent of the American population may have suboptimal manganese status. At the same time, concern has been raised for possible adverse effects resulting from excessive intakes of manganese, especially from drinking water. Determination of a safe and adequate intake of manganese is made difficult for many reasons, including: 1) there are no good biological indicators of manganese exposure; 2) the bioavailability of ingested manganese is highly variable between individuals and may be affected by many host factors, as well as other dietary constituents; and 3) toxicity studies by the oral route have been conducted primarily in rodents, which are not a useful model for quantitative risk assessment. While very little is known about manganese toxicity to humans following ingestion, inhalation exposure reveals a long latency period, with effects mimicking those of other neurologic diseases associated with aging. There also appears to be a dichotomy in human sensitivity to manganese, with 1–2% of the population being more susceptible. Analysis of the complete database for manganese indicates a likelihood that an overlap may exist between intakes that are beneficial to some individuals yet toxic to others. The setting of environmental regulations, then, requires a careful balancing act of these conflicting factors.

941 THE REFERENCE DOSE FOR ZINC

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Zinc supplementation can effect copper absorption and superoxide dismutase activity. Decreases in serum copper were noted in both males and females exposed to daily use of 50 mg zinc/day supplements for periods of 6 to 10 weeks. Parallel decreases in erythrocyte superoxide dismutase activity potentially weaken physiological defenses against free radical oxygen. Data on the long term effects of zinc supplements on copper homeostasis are not available but case study data support long term adverse effects of supplemental zinc (50–200 mg/day) on copper bioavailability. Based on these data, 50 mg/kg/day supplemental zinc is a minimal LOAEL for effects on human health. Using standard EPA risk assessment practices, a Reference Dose (RfD) of 0.3 mg/kg/day can be derived which provides adequate zinc to meet essential dietary needs (RDA) for all age/sex groups except infants and children while protecting susceptible adults from potential adverse effects. Support for the zinc RfD is provided by data that indicate possible adverse effects of zinc supplementation (50–300 mg/day) on serum high density lipoprotein concentrations (a risk factor for cardiovascular disease). Adverse effects of short term zinc supplementation on serum magnesium and iron concentrations have also been reported.

942 REFERENCE DOSES FOR TRIVALENT AND HEXAVALENT CHROMIUM: NUTRITIONAL BENEFIT VERSUS TOXICITY

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In its trivalent form, chromium is an essential element. It has multiple physiological roles, the primary one being insulin potentiation. The Estimated Safe and Adequate Daily Dietary Intake (ESADDI) for chromium is 50–200 ug. The general US population, however, typically consumes even lower levels (25–33 ug/day), so that marginal chromium deficiency is not uncommon. Trivalent chromium (CrIII) is relatively nontoxic, as reflected by a Reference Dose (RFD) of 1 mg/kg/day, an intake 350 times higher than the high end of the ESADDI. It should be noted, however, that this RfD is for insoluble salts of CrIII, which are likely to be less bioavailable than chromium compounds found in the diet. Concern for chromium toxicity is due to the hexavalent form, which has industrial uses. Toxicity has been well-established following inhalation and dermal exposures to hexavalent chromium (CrVI), but is less well-characterized following ingestion. The stomach has a fairly large reducing capacity such that ingested CrVI is reduced to CrIII prior to absorption. Only if the reducing capacity of the stomach is exceeded would toxicity be expected to result. The RfD for CrVI is significantly lower than that for CrIII, with a value of 0.005 mg/kg/day. This RfD, however, is based on a 1-year drinking water study in rats which reported no adverse effects at doses of 2.4 mg/kg/day. It appears, therefore, that CrVI poses more of a hazard from inhalation or dermal exposures than it does following ingestion.

943 RISK ASSESSMENT OF SELENIUM: A REVIEW OF THE REFERENCE DOSE (RfD) AND THE RECOMMENDED DIETARY ALLOWANCE (RDA)

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Selenium (Se) intakes above 19 (male) and 14 μg/day (female) are the minimum intakes of selenium that prevent selenium deficiency and Keshan disease, a condition predisposed by Se deficiency. A human physiological requirement of approximately 40 μg Se/day was established by supplementing adult Chinese males of low selenium status with selenomethionine. From these data and accounting for body weight and individual variation in the North American adult male and female, an RDA of 0.87 μg/kg for Se was

determined by the NAS. Likewise the USEPA's RfD (Poirier and Foreman, 1991) is based on a population of approximately 400 Chinese subjects in different seleniferous areas (Yang et al., 1989). The daily average Se intakes were estimated from an algorithm based on blood Se levels. In the adult they are 70, 195, and 1438 μg (males) and 62, 198, and 1238 μg (females) in the low-, medium-, and high-selenium areas, respectively. A marginal safe level of daily selenium intake was estimated from serum selenium levels in subjects that did not exhibit any biochemical or morphological signs of selenosis. This blood Se level corresponded to a daily selenium intake of 863 μg and is designated as the NOAEL. A sensitive subpopulation (5/349 adults) showed persistent clinical signs of selenosis with a calculated intake of 1,261 μg Se/day. This level of Se intake was identified as the LOAEL. A study in 142 volunteers from seleniferous areas in the U.S. support the Chinese findings (Longnecker et al. 1991). Application of the uncertainty factor of 3 for intraspecies extrapolation to account for sensitive individuals yields an RfD for Se and compounds of 0.005 mg/kg/day. The RfD for Se as currently defined (350 μg/day) provides a 5 fold margin of safety above the adult RDA (70 μg/day). Thus for Se, the RfD provides a high confidence of protection against chronic toxicity without impinging upon the RDA. These data demonstrate that the RfD paradigm can successfully estimate safe levels of intakes of an essential trace element.

944 NON-CANCER RISK ASSESSMENT FOR ESSENTIAL METAL: COPPER

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The U.S. EPA methodology for development of Reference Dose (RfD) involves the determination of NOAEL/LOAEL from human/animal data. Application of one or more uncertainty factor(s) to this number results in an RfD for the chemical of concern. A methodological paradox arises with essential trace metals because the RfD is expected to be protective against any ill- health, but the derived RfD is below the RDA; thus causing concern for nutritional deficiency. The NAS-NRC recommended levels for copper required for adequate nutrition is 2–3mg/day; in contrast, however, using the EPA paradigm for RfD, a human LOAEL (0.076 mg/kg/day, Chuttani et al., 1965) or an animal NOAEL (0.85 mg/kg/day, Jenkins and Hidiroglou, 1989) could possibly yield an RfD that is lower than the ESADDI. This paper will discuss this dilema first by presenting the current RfD paradigm, followed by an approach that will allow human exposure to lie between essentiality and toxicity. (This abstarct does not reflect EPA Policy.)

945 USES OF THE REFERENCE DOSE (RfD) AND RECOMMENDED DIETARY ALLOWANCES (RDAs) FOR ESSENTIAL TRACE ELEMENTS (ETEs)

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RfDs and RDAs are established for different purposes. The EPA uses the RfD for environmental chemical regulation, while the Food and Nutrition Board of the National Academy of Sciences establishes RDAs as nutritional guidelines for ETEs. Recently, however, EPA has been required to regulate various ETEs. For this purpose, EPA used the noncancer effects of ETEs to derive RfDs. The RfD is based on the concept that an organism can tolerate "a range of exposures from zero to some finite value..." The RfD has proven to be useful for most noncarcinogenic chemicals, but ETEs are unusual; zero or less than adequate exposure could result in deficiency. Therefore, for ETEs, EPA adjusted its methodology to consider the deficiency state. To obviate the possibility of verifying a value that could lead to a deficient state, EPA has used the RDA as a lower limit for the RfD. This permitted EPA to set RfDs for ETEs that consider both the essential and toxic effects of these chemicals. Examples of these changes will be given.

946 CHRONIC ORAL TOXICITY VALUE FOR IRON

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While chronic iron toxicity can occur in people that have genetic metabolic disorders resulting in excessive iron absorption, abnormal hemoglobin synthesis or who receive frequent blood transfusions, there is a long-standing controversy as to whether a chronic overload from oral intake is possible in individuals that have a normal ability to control iron absorption. Moreover, human epidemiologic studies and clinical case studies do not delineate a precise NOAEL/LOAEL boundary for chronic oral toxicity in normal individuals consuming western diets because of confounding factors, inadequate endpoint assessment, insufficient exposure durations or inadequate number of subjects. Nonetheless, it is possible to establish a NOAEL for chronic iron overload based on the distribution of dietary intakes and biomarkers of iron status (i.e., serum ferritin and transferrin saturation) as captured in national surveys such as NHANES. This NOAEL should represent a chronic daily intake in the U.S. population that is both sufficient to protect against iron deficiency and insufficient to cause toxic effects of iron overload in people with normal iron metabolism. The NOAEL can be compared to RDAs and less robust estimates of human AELs to estimate the NOAEL/LOAEL boundary or intake range that might encompass the boundary. This approach was explored in a case study using an analysis of NHANES II data by Looker et al. (1988). A NOAEL of 0.27 mg/kg/day was estimated; an uncertainty factor of 1 was applied to derive an RfD of 3E-1 mg/kg/day. This RfD may not protect against iron toxicity in people that have disorders of iron metabolism and could be overly conservative if used to assess risks associated with exposures to forms of iron that have low bioavailability. (This abstract does not necessarily reflect U.S. EPA policy.)

947 THE EFFECTS OF MANGANESE ON ANTIOXIDANT ENZYMES IN DIFFERENT REGIONS OF RAT BRAIN

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Manganese (Mn) is one of the essential trace elements required for all living organisms, however, at higher concentrations it produces neurological disorders. Recently it has been suggested that Mn-induced neurotoxicity is mediated via oxidative stress generated by free radicals. The present study was designed to investigate the effects of Mn exposure on the activities of antioxidative enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) and reduced glutathione (GSH) content in different brain regions of male CD-1 rats. Superoxide scavenging properties of $MnCl_2$ were also demonstrated *in vitro* and it was found that Mn scavenges superoxide in a dose-dependent manner. The activity of total SOD did not vary significantly in any region of brain exposed to Mn, however, a significant increase of Mn-SOD activity was observed in the hippocampus, cerebellum and brain stem. No significant effect of Mn on GPx activity was observed in any region of the brain. GSH content was significantly reduced in cerebellum but no changes were observed in other regions. In conclusion the results show that the exposure of rats to Mn produces significant effects on Mn-superoxide dismuatse activity.

948 MPP^+-INDUCED NEUROTOXICITY IN MOUSE IS AGE-DEPENDENT AS EVIDENCED BY THE SELECTIVE INHIBITION OF ELECTRON TRANSPORT COMPLEXES

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1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine ($MPTP^+$) has been reported to cause selective neurotoxicity by inhibiting complex I in mitochondria via its toxic metabolite, 1-methyl-4-phenylpyridine (MPP^+) which is formed during the bioactivation of MPTP by monoamine oxidase B. In this report the effect of MPP^+ on the four mitochondrial respiratory chain complexes has been evaluted by incubating brain mitochondria from mice of different ages with MPP^+ (200 μM) and monitoring enzyme activities of complexes I, II, III, and IV at 5,10,15,30,60 and 120 minutes. Complexes I, III, and IV showed significant inhibition within 15 minutes in all the age groups studied, followed by some recovery in enzyme activities upon further incubation for complexes I and IV. However, complex II was not affected by MPP^+ at any age. Our data suggest that inhibition of complexes I, III and IV by MPP^+ effectively restricts the transport of electrons down the respiratory chain, which ultimately leads to decreased ATP production. This could further aggravate the effects of oxidative stress as ATP is required for the synthesis of glutathione (GSH), one of the important scavengers of free radicals. In this study, inhibition was more severe in mitochondrial preparations from older rather than younger mice. Additionally, young animals showed faster recovery following inhibition than old animals for complex I. Impaired respiratory chain function in older animals compared to younger ones supports the hypothesis of accumulation of age-related mitochondrial DNA mutations which partly encode for subunits of complexes I, III, and IV. These results indicate that inhibition of complexes I, III and IV may be the underlying cause of neurotoxicity and

that this effect is intensified by age-associated dysfunction of electron transport.

949 EFFECTS OF MANGANESE ON INOSITOL POLYPHOSPHATE (InsP) RECEPTORS AND NITRIC OXIDE SYNTHASE (NOS) IN RAT CEREBELLUM

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Manganese (Mn) is an essential trace element, however, at high doses it causes neurotoxicity in rodents and Parkinson's disease like symptoms in humans. The neurotoxic effects of Mn include degeneration of dopaminergic neurons, impairment of energy metabolism and perturbation of phosphoinositide hydrolysis resulting in altered Ca^{+2} homeostasis. Nitric oxide synthase (NOS) is a Ca^{+2}-dependent cytosolic enzyme involved in the biosynthesis of nitric oxide which mediates several neuronal events. This study was designed to assess the effects of $MnCl_2$ on Ca^{+2}-dependent nitric oxide synthase (NOS) and $InsP_3$ and $InsP_4$ receptors in rat cerebellum. Mn at concentrations of 5–100 uM significantly decreased (9–62%) ^{3}H-$InsP_3$ binding, whereas at 100– 400 μM, Mn increased (7–1, 321%) ^{3}H-$InsP_4$ binding to cerebellar membranes in a concentration-dependent manner. Also, $MnCl_2$ *in vitro* (100–1000 μM) and *in vivo* (2.5 and 5.0 mg/kg administered for 30 days) inhibited NOS activity in cerebellum. This data suggest that Mn alters InsP-mediated intracellular Ca^{+2} mobilization and NO production by inhibiting NOS activity resulting in neuronal dysfunction. (Supported by ORISE through an Interagency agreement between the DOE and NCTR).

950 THE EFFECT OF ALCOHOL ON INTRACELLULAR CALCIUM MOBILIZATION IN GLIAL CELLS

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Glial cells play an important role in brain development. Our lab has recently demonstrated that ethanol inhibits muscarinic receptor-mediated proliferation of glial cells. Since calcium is involved in cellular proliferation, this study was undertaken to investigate the effects of ethanol on intracellular calcium mobilization caused by muscarinic receptor stimulation in human 132 1N1 astrocytoma cells and in rat primary cortical astrocytes. Intracellular calcium was measured using the calcium sensitive probe Indo-1, on an ACAS (attached cell analysis and sorting) instrument. Carbachol caused a concentration-dependent (atropine sensitive) spike in intracellular calcium, followed by oscillations and a sustained elevation of calcium in both cell types, with more heterogeneity and oscillations in the primary cells. The initial calcium spike occurred in the absence of extracellular calcium, however, the duration of the sustained elevation was decreased, indicating a role for intracellular and extracellular calcium for the two phases, respectively. Ethanol exhibited a concentration-dependent inhibition of the carbachol-mediated response. In the presence of extracellular calcium, ethanol caused a reduction in the spike size, but did not affect the percentage of cells responding. Studies are currently being carried out to determine the specificity and mechanism of this ethanol effect. (Supp. in part by grant AA-08154 and a grant from the ADAI, Univ. of Washington).

951 CYCLOSPORIN A DELAYS MITOCHONDRIAL DEPOLARIZATION INDUCED BY NMDA IN CORTICAL NEURONS: EVIDENCE OF THE MITOCHONDRIAL PERMEABILITY TRANSITION

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NMDA causes a rapid increase in intracellular Ca^{2+} leading to collapse of mitochondrial membrane potential ($\Delta\Psi$) and eventually cell death in cortical neurons. The aim of this study was to investigate the cellular mechanisms responsible for mitochondrial depolarization using laser scanning confocal microscopy of single cultured rat cortical neurons. To monitor $\Delta\Psi$, neuronal mitochondria were labeled with tetramethylrhodamine methylester and loss of cell viability was assessed by propidium iodide uptake into nuclei. In the presence of 500 μM NMDA, 50% of the neurons showed mitochondrial depolarization in 15 min (n = 28 cells). Cyclosporin A, an inhibitor of the mitochondrial permeability transition, delayed depolarization in a dose-dependent manner (0.2–1 μM). In the presence of 1 μM cyclosporin A, mitochondrial depolarization in 50% of neurons did not occur until 43 min (n = 23 cells). Loss of cell viability was not observed during the time course of the experiments. In conclusion, these data show for the first time that mitochondrial depolarization induced by NMDA may be due to the induction of the mitochondrial permeability transition.

952 INVOLVEMENT OF CA2+/CALMODULIN-DEPENDENT PROTEIN KINASE II IN CYANIDE-INDUCED CYTOTOXICITY IN CULTURED CEREBELLAR GRANULE CELLS

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Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) is highly enriched in neural tissues and is a multifunctional enzyme involved in neuronal intracellular signalling. CaM kinase II activity is elevated in ischemia and glutamate-induced cell death. Moreover, it has been shown that a CaM kinase II inhibitor can protect cortical neurons from NMDA or hypoxia-induced cell death. In this study, the involvement of CaM kinase II in cyanide-induced neurotoxicity was investigated in rat primary cultured cerebellar granule cells. KN62 (5 μM), a specific CaM kinase II inhibitor, significantly decreased KCN-induced LDH release by 46%. Since increased $[Ca^{2+}]_i$ plays an important role in cyanide-induced neurotoxicity, the role of CaM kinase II in the regulation of $[Ca^{2+}]_i$ in cyanide treated cells was also studied. KCN (1 mM) generated a 291.2 ± 24.9 nM increase of $[Ca^{2+}]_i$, and this effect was decreased 65% by pretreating cells with KN62 (5 μM) for 30 min. Pretreatment with MK801 (1 μM) blocked the KCN-induced increase of $[Ca^{2+}]_i$. In comparison, KN62 could not block the increase of $[Ca^{2+}]_i$ generated by glutamate (50 μM) or by a low dose of KCN (50μM), although KN62 decreased by 26% the elevation of $[Ca^{2+}]_i$ induced by application of NMDA (50 μM). These studies indicate that CaM kinase II is activated during cyanide-induced chemical hypoxia in cultured cerebellar granule cells and plays a role in cyanide-induced cytotoxicity. Activated CaM kinase II participates in the alteration of $[Ca^{2+}]_i$ possibly indirectly by increasing the release of glutamate, which then stimulates the influx of Ca^{2+} via the NMDA receptor. (Supported by NIH Grant ES04140).

953 INHIBITION OF REGULATORY VOLUME DECREASE (RVD) IN RAT ASTROCYTE CULTURES BY METHYL-MERCURY (MeHg) IS DUE TO INCREASED Na^+ UPTAKE

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Neonatal rat primary astrocyte cultures were swollen by exposure to hypotonic buffer ± 10 μM MeHg. The effects of MeHg on K^+ (^{86}Rb), taurine, D-aspartate and Na^+ fluxes, and RVD were measured with an electrical impedance method, coupled with on-line measurements of efflux of radioactive ions and amino acids. Addition of 10 μM MeHg significantly ($p<0.001$) inhibited RVD in swollen astrocytes. MeHg also significantly ($p<0.0001$) increased ^{86}Rb release, and significantly ($p<0.001$) decreased ^{3}H-taurine release. MeHg had no effect on ^{3}H-D-aspartate release from swollen astrocytes. There was a significant ($p<0.001$) increase in $^{22}Na^+$ uptake in cells exposed to MeHg (537.2 ± 26.8 ng Na^+/mg protein) compared to cells exposed to hypotonic buffer (124.8 ± 20.42 ng/mg protein). K^+ release for astrocytes exposed to MeHg and hypotonicity equaled 252.4 ± 46.9 ng K^+/mg protein. When Na^+ in the hypotonic buffer was replaced with N-methyl-D-glucamine, RVD in the presence of MeHg was indistinguishable from controls. These results indicate that MeHg-induced inhibition of RVD occurs in the presence of increased K^+ release. In order for RVD to be inhibited by MeHg, another osmolyte must enter into the astrocytes in sufficient quantity to counteract the loss of K^+. Accordingly, it is postulated that the combined effect of increased Na^+ influx and taurine retention leads to the MeHg-induced inhibition of RVD in swollen astrocytes. Supported by NS 23750 [HKK]& US EPA R-819210 (MA).

954 EFFECTS OF METHYL MERCURY ON THE ACTIVITY OF MONOAMINE OXIDASE IN BRAIN SYNAPTOSOMES OF RATS AND RABBITS AND IN RABBIT AND HUMAN PLATELETS *IN VITRO*

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Monoamine oxidase (MAO; EC 1.4.3.4) is an enzyme considered to have an important role in the regulation of the levels of biogenic amines in the brain and peripheral tissues. Methyl mercury (MeHg) is known to inhibit the uptake and release of dopamine (DA), noradrenaline (NA) and serotonin (5-HT) in rat brain synaptosomes. It is also known that circulating platelets represent an excellent model for an easy assessment of the effect of MAO-B inhibitors in extracerebral tissue. The present study was carried out to determine the effects of MeHg on the activity of MAO in vitro in synaptosomes of different brain regions of rabbits and rats as well as in rabbit and human blood platelets. The MAO activity was assessed by a fluorometric method using kynuramine as substrate for both MAO-A and MAO-B. MeHg pretreatment inhibited the activity of MAO in the synaptosomes of cortex, hypothalamus, hippocampus, striatum, brain-stem and cerebellum of both male New Zealand rabbits and Sprague-Dawley rats in a concentration-dependent manner. The threshold concentration of MeHg required to significantly inhibit the MAO activity in these different brain regions of rabbits and rats was found to be same, i.e. 1μM, except for rat striatum it was 2.5μM. The IC_{50} (MeHg) for rat brain synaptosomes were: 2.4μM for cortex, striatum, hypothalamus, cerebellum and brainstem and 1.9μM for hippocampus, whereas for rabbit brain synaptosomes, these IC_{50} (μM) were: 3.9 (cortex), 2.3 (striatum), 1.7 (hypothalamus), 3.0 (cerebellum), and 3.2 (brainstem). The threshold and IC_{50} for MeHg for MAO activity in rabbit platelet-rich plasma were 50 and 225μM respectively, whereas for humans these were 20 and 23μM respectively, suggesting that human MAO activity is more sensitive to the action of MeHg than that of rabbit. (Supported by MRC, Hydro-Quebec, CEA)

955 INTERACTIONS OF LEAD WITH ZINC FINGER PROTEINS

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Recent studies have shown that *in vivo* exposure to lead (Pb) modulated the mRNA levels of developmental markers in the brain. Interferences with the regulation of differential gene expression may be due to perturbation of transcription factors such as zinc finger proteins (ZFP), which play key roles during growth/differentiation. Using the gel shift mobility assay and Northern analysis, we examined if the addition of Pb to nuclear extracts from HeLa cells or the presence of low Pb concentrations in the media of differentiating PC12 cells, would alter the DNA binding or mRNA expression of ZFP. We found that low concentrations of Pb (<1.0 mM) produced a dramatic enhancement of Sp1 DNA binding, while higher concentrations of Pb (>5 mM) abolished the sequence specific DNA binding activity of Sp1 in nuclear extracts derived from HeLa cells. In PC12 cells, a marked Sp1 DNA binding activity was prematurely observed half an hour after exposure to Pb (0.1 μM). At the same time, a more pronounced expression of zif268 mRNA was also seen in these same cells. These studies suggest that exposure to Pb may alter the DNA-binding as well as the expression of ZFP *in vitro*.

956 CHARACTERIZATION OF INTERACTION OF METHYLMERCURY (MeHg) WITH Ca^{2+} CHANNELS OF RAT CEREBELLAR SYNAPTOSOMES

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The interaction of MeHg with neuronal Ca^{2+} channels in rat cerebellar synaptosomes was studied using radiotracer influx assay. Influx of $^{45}Ca^{2+}$ was measured after 1 sec K^+ -induced (41.25mM) depolarization. The objective was to determine the efficacy of MeHg in blocking Ca^{2+} channels of cerebellum, a prominent target of MeHg neurotoxicity. MeHg blocked $^{45}Ca^{2+}$ influx in a concentration-dependent manner; the estimated IC_{50} is 125 μM. At 250 μM, MeHg reduced $^{45}Ca^{2+}$ influx by approximately 64%. Similar results were obtained in previous studies using cerebrocortical synaptosomes. To identify the specific Ca^{2+} channel subtypes that MeHg may act on to block $^{45}Ca^{2+}$ influx, several Ca^{2+} channel blockers, which interact with pharmacologically-distinct classes of channels were used. These included nifedipine (Nif, L-type), ω-conotoxin GVIA (ω-CgTx GVIA, N-type), ω-conotoxin MVIIC (ω-CTx MVIIC, P, Q, and perhaps R-types), and ω-agatoxin IVA (ω-Aga IVA, P type). Nif at concentrations up to 10 μM had no effect on $^{45}Ca^{2+}$ influx in either control or MeHg-treated groups. ω-CTx MVIIC totally blocked $^{45}Ca^{2+}$ influx at 5 μM; its IC_{50} was approximately 0.5 μM. Partial block of $^{45}Ca^{2+}$ influx was observed with ω-CgTx GVIA and ω-Aga IVA; the maximum percent block was 35% (5 μM ω-CgTx GVIA) and 33% (250 nM ω-Aga IVA), respectively. Pretreatment with either ω-Aga IVA or Nif did not alter the effect of 100 μM MeHg on $^{45}Ca^{2+}$ influx, however ω-CgTx GVIA and ω-CTx MVIIC both partially blocked the effects of MeHg. These results indicate that multiple Ca^{2+} channel subtypes coexist in cerebellar synaptosomes, and MeHg interacts with ω-CgTx GVIA- and ω-CTx MVIIC-sensitive Ca^{2+} channel subtypes to block $^{45}Ca^{2+}$ influx. Supported by NIEHS grant ES03299.

957 PURIFICATION OF SYNAPTOSOMAL Zn^{2+}-BINDING PROTEINS WHICH ARE SENSITIVE TO METHYLMERCURY

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Methylmercury elevates the free intrasynaptosomal concentration of Zn^{2+} ($[Zn^{2+}]_i$) upon acute exposure. This effect is believed to result from release of endogenous Zn^{2+} from synaptosomal proteins. The goal of this study is to identify the synaptosomal zinc-binding proteins which contribute to the MeHg-induced elevation in $[Zn^{2+}]_i$. Synaptosomal protein extracts were prepared from rat cortical synaptosomes using 50 mM EPPS (pH 7.4) containing 0.1 M NaCl. The soluble protein fraction was recovered by centrifugation, and assayed for MeHg-induced Zn^{2+} release using the fluorescent cation indicator fura-2. Addition of MeHg to the soluble fraction caused a shift in the fura-2 excitation spectrum consistent with an increase in free Zn^{2+} concentration. The soluble protein extract was fractionated using DEAE-cellulose chromatography, and a stepwise elution gradient of 0.2, 0.5 and 2.0 M NaCl. MeHg-induced Zn^{2+} release activity eluted primarily in the 0.5 M NaCl fraction as determined by shifts in the fura-2 excitation profile following MeHg addition. Further purification of this active fraction by Sephadex G200 gel filtration chromatography yielded an active peak with an apparent molecular weight between 50 to 70 kDa. Future efforts will be directed toward purification and identification of the MeHg-sensitive Zn^{2+}-binding protein(s) present in this fraction. Supported by NIH grant ESO3299 and a grant from the Research Excellence Fund from Michigan State University.

958 DEVELOPMENTAL TOXICANT INDUCED CHANGES IN GENE EXPRESSION: ROLE IN TOXICITY IN VITRO

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A major objective of our research is to understand the significance of developmental toxicant induced changes in gene expression. Towards that goal, we have evaluated genes that were differentially expressed under conditions of developmental toxicity *in vitro*. We utilized the model teratogen and well characterised **DNA** alkylating agent, ethylnitrosourea (**ENU**), in our primary **CNS** cultures to generate a pool of 22 **cDNAs** whose expression changed following exposure. These were identified using a modification of the Differential Display technique that uses 20-mers. These **cDNAs** were screened against digoxygenin-labeled total cDNA using slot blot analysis to confirm our initial observations that a subset increased or decreased following toxicant exposure. Several of the **cDNAs** whose expression changes were confirmed on slot blots were cloned, sequenced and analyzed on Northern blots. Two of these exhibited >90% homology with previously identified genes found in GenBank. These include **GATA**-3 transcription factor and an isoform of non-muscle tropomyosin. GATA-3 has previously been implicated in neuronal differentiation. The time course during 5 days of culture (equivalent to days 12–17 *in vivo*) of expression of these genes during normal differentiation as well as following exposure to the developmental toxicant was determined. Results of these studies demonstrated the importance of evaluating genes previously unassociated with developmental toxicity. Supported by **NIH ES-03157** and **ES-07032**.

959 CYANIDE STIMULATES REACTIVE OXYGEN SPECIES GENERATION THROUGH THE CYCLOOXYGENASE/ LIPOXYGENASE PATHWAYS: IMPLICATION FOR CYTOTOXICITY

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KCN indirectly activates NMDA receptors to simultaneously generate nitric oxide (NO) and reactive oxygen species (ROS). In this study the mechanism of ROS generation and its relationship to cell injury was determined in cerebellar granule cells. Intracellular levels of NO and ROS were determined by use of the oxidant-sensitive fluorescence dye, 2,7-dichlorofluorescin. Pretreatment of cells with chelerythrine (CHE) (PKC inhibitor) or quinacrine (QUI) (PLA_2 inhibitor) prior to KCN (100 μM) partially blocked ROS generation, whereas co-incubation increased the block by ~75%. Pretreatment with L-NAME (NO synthase inhibitor) or SOD/catalase with QUI or CHE produced additional attenuation of NO and ROS generation, suggesting involvement of PKC. Additionally, cells were treated with indomethacin (INDO) or eicosatetraynoic acid (ETYA), which inhibit cyclooxygenase (COX) and lipoxygenase (LOX), enzymes that metabolize arachidonic acid (AA). Both inhibitors attenuated (~35%) KCN-induced ROS generation. Complete block (>90%) of oxidant species generation was noted when L-NAME was combined with either INDO or ETYA. Furthermore, a correlation was seen between NO and ROS formation and lipid peroxidation (malonaldehyde). Pretreatment with CHE, QUI or INDO partially blocked MDA generation, whereas co-incubation of L-NAME with either QUI, INDO or ETYA decreased by 80–90% MDA formation. In cytotoxicity studies, KCN-induced cell death was partially blocked by CHE, QUI or INDO, but significant protection from cytotoxicity was observed when L-NAME was combined with these compounds. It is concluded that the activation of PLA_2 and in turn the COX/LOX pathways contribute significantly to KCN-induced ROS production. Inhibition of COX/LOX and NOS protects against KCN-induced lipid peroxidation and cytotoxicity. (Supported by NIH grant ES04140).

960 ALTERED NEURITE OUTGROWTH IN DIFFERENTIATED EMBRYONAL CARCINOMA CELLS EXPOSED TO 2,5-HEXANEDIONE

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Pluripotent embryonal carcinoma cells (EC) differentiate into neurons after exposure to retinoic acid (RA), as identified by morphology and the expression of low and medium weight neurofilament (NF) proteins which are first observed 4 d after RA treatment. Western blots and immunofluorescence were used to identify the appearance of the high molecular weight neurofilament (NFH) protein subunit 6 d after RA treatment. The delay in NFH expression corresponds to the late appearance of this protein during neuronal development *in vivo*, validating the EC model for use in the study of neurite development. This model was used to examine the effects of the neurotoxic γ-diketone, 2,5-hexanedione (HD), a specific NF toxicant, on neurite initiation and extension. EC cells grown for 4 or 9 d following RA treatment were exposed to 3 or 6 mM HD, or to 6 mM acetone for 48 h. Acetone-treated cells were indistinguishable from controls at both time points. Cells treated with HD at day 4 of differentiation developed fewer neurites, and those that did develop were shorter than neurites from control cultures. Neurites from cells treated with HD on day 9 appeared retracted and were thicker than neurites from control cultures. The effects of HD on neurite morphology were dose-dependent. These data suggest that HD-induced alterations in NF proteins inhibit neurite initiation during the early stages of differentiation and cause retraction of pre-existing neurites during later stages of differentiation. (Supported by a Johnson & Johnson Developmental Neurotoxicology Scholar Grant)

961 PHOSPHORYLATION OF MEMBRANE PROTEINS IN ERYTHROCYTES TREATED WITH LEAD

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In several types of cells, exposure to lead results in an increase in the level of protein kinase C in membranes. In this presentation, we extended these studies to human erythrocytes and, in addition, studied the phosphorylation of membrane proteins. A significant increase in the phosphorylation of membrane cytoskeletal proteins, adducin, and proteins 4.1 and 4.9 was observed in human erythrocytes treated for 60 min with lead acetate at concentrations greater than 100 nM. These same proteins were phosphorylated when erythrocytes were treated for 10 min with 50 nM phorbol 12-myristate 13-acetate (PMA). Similarly, protein kinase C activity was elevated and an increase in the amount of protein kinase C alpha was observed in membranes from erythrocytes exposed to concentrations of lead acetate above 100 nM. Phosphorylation of these membrane cytoskeletal proteins stimulated by lead acetate or by PMA was not observed in erythrocytes depleted of protein kinase C. Finally, no change in levels of calcium or diacylglycerol was observed in erythrocytes stimulated with 100 nM lead acetate. These results show that in erythrocytes lead acetate stimulates the phosphorylation of membrane cytoskeletal proteins by a mechanism dependent on protein kinase C. Since levels of calcium or diacylglycerols did not increase, it appears that lead may activate the enzyme by a direct interaction.

962 EFFECTS OF Cd, Zn AND Al ON THE GENERATION OF REACTIVE OXYGEN SPECIES: AN IN VITRO STUDY

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Reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anion and hydrogen peroxide are known to cause cell damage. Recently we have reported that metals such as Hg, Mn and Fe can induce the generation of reactive oxygen species (ROS) in cerebral tissue. The present study is designed to evaluate whether other metals such as Cd, Zn and Al also produce ROS in cerebral tissue. Cd is known to produce nephrotoxicity and cardiotoxicity and is also a suspected carcinogen. It is still unclear, however, whether Cd, Zn and/or Al produce neurotoxicity via oxidative stress. Rat brain regions [caudate nucleus (CN), frontal cortex (FC), hippocampus (HIPP) and cerebellum (CE)] were used to evaluate the *in vitro* effects of these metals on the production of ROS. 2,7-dichlorofluorescein diacetate (DCFH-DA), a molecular probe, was used to quantitate ROS. Zn (1–50μM) and Cd (1–1000μM) produced a dose-dependent increase in ROS in CN, FC, HIPP and CE of rat brain. Al at similar concentrations did not show any significant increase in ROS. These *in vitro* studies suggest that Zn and Cd induces oxidative stress.

963 INDUCTION OF STRESS PROTEINS BY DIETHYLDITHIOCARBAMATE (DDC) AND/OR COPPER CHLORIDE IN RAT HIPPOCAMPAL ASTROCYTES

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Disulfiram (Antabuse), a potent chelator and its principal metabolite diethyldithiolcarbamate (DDC) have been implicated in central neurotoxicity via the redistribution of metals to the brain. Recently, our laboratory has demonstrated that rats treated with disulfiram show changes associated with oxidative stress in direct relationship to the accumulation of copper in the cerebellum and the hippocampus. The presence of heat shock proteins (hsp) have been used to demonstrate alterations in cellular activity and oxidative damage resulting in cellular degeneration. Hsp 72kD is the most highly inducible form in the hsp70 family and its induction is related to cellular insult. Hsp 32kD (HO -1) is the only heme oxygenase isoenzyme, between HO -1 and HO -2, which is a stress protein. It is the only hsp whose induction is associated with oxidative damage, resulting from heme, metals, hormones, sulfhydryl depleting agents and UV radiation. The purpose of this investigation was to look at the stress protein response of rat hippocampal astrocytes to DDC and/or copper via western blot analysis and metabolic labeling with ^{35}S-methionine. Western blot analyses show induction of hsp32 and hsp72 after DDC and copper treatment. An additive effect is seen when DDC and copper are combined. The induction of hsp is *denovo* synthesis as suggested by the metabolic labeling. Identification of target proteins, such as hsp32kD and hsp72kD, can help us elucidate the mechanism of toxicity and improve our understanding of cellular responses to metals and their implications in stress tolerance and oxidative damage.

964 FUMONISIN B1 (FB1) INCREASES 2', 3'-CYCLIC NUCLEOTIDE 3'-PHOSPHOHYDROLASE (CNP) ACTIVITY IN PRIMARY CULTURES OF RAT CEREBRUM: IMPACT ON OLIGODENDROCYTES

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Chronic dietary consumption of the mycotoxin FB1 is associated with leukoencephalomalacia, but identification of the cellular mechanism underlying this neurotoxicity is difficult to determine because of concurrent adverse

systemic changes. The present investigation used an in vitro approach to assess the short term consequences of direct FB1 exposure on glia in the absence of potentially confounding systemic factors. Subconfluent cultures of postnatal rat cerebra were administered graded concentrations of FB1 (0.5 to 75 μM) beginning at 5 days in vitro (DIV), and the cultures were morphologically and biochemically evaluated at 10 and 15 DIV. At these ages, phase-contrast photomicrographs coded for unbiased analysis revealed that exposure to FB1 increased the numbers of oligodendrocyte-like cells in the primary cultures. Levels of sphingolipid-associated constituents, sphingosine (So) and sphinganine (Sa), were determined by HPLC. FB1 exposure resulted in significant increase of both Sa and Sa/So ratios at 10 and 15 DIV. Fluorescent spectrophotometric assay of the activity of CNP, an enzyme associated with myelin and oligodendrocytes, showed that FB1 exposure increased CNP activity at 10 and 15 DIV. No cytotoxicity was observed. These correlated morphological and biochemical results suggest that short-term exposure to FB1, in the absence of systemic variables, enhanced the proliferation, survival or differentiation of oligodendrocytes.

965 GABA$_A$ RECEPTOR ASSOCIATED CHLORIDE FLUX IN RATS RENDERED TOLERANT TO OR DEPENDENT ON PENTOBARBITAL

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The *in vitro* receptor-stimulated synaptoneurosomal chloride uptake induced by pentobarbital or muscimol was studied in the brains of rats which had been rendered tolerant to or dependent on pentobarbital. In the chronic study, rats received continuous administration of sodium pentobarbital by intracerebroventricular (i.c.v.) infusion. In rats continuously infused with pentobarbital for 6 days or in rats terminated the infusion for 24 hr, the basal synaptoneurosomal chloride uptake was not altered in either the cerebral cortex or the cerebellum. On the other hand, pentobarbital (500 μM)-stimulated chloride uptake was significantly decreased in both cerebral cortices and cerebella of rats which received pentobarbital for 6 days as compared with the saline control groups. Twenty-four hours after termination of pentobarbital infusion, this high concentration of pentobarbital stimulated chloride uptake remained low in both cerebral cortices and cerebella of rats which had been infused with pentobarbital for 6 days. In addition, similar results were also obtained in muscimol (2.5 μM)-stimulated chloride uptake in the cerebral cortices of pentobarbital infused animals. However, pentobarbital infusion failed to alter muscimol-stimulated chloride uptake in the cerebellum. These results suggest that the GABA$_A$ receptor regulated chloride uptake is down regulated after chronic pentobarbital administration. The results also suggest that down regulation of the GABA$_A$ receptor chloride channel complex takes place at different recognition sites on the complex. It further substantiates the allosteric effects of GABA and barbiturate recognition sites. (Supported by NIDA-04480).

966 EVIDENCE OF ASTROGLIA AS A CNS TARGET OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN

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This paper reports the results from in vitro experiments utilizing vital fluorescent probes and biochemical assays to examine the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in primary rat astroglia in an effort to identify the cellular site(s) involved in toxicity. Application of 100 nM TCDD resulted in altered astroglial Ca^{2+} channel activity, a dramatic decrease in GSH, a disrupted mitochondrial membrane potential, a significant decrease in GS activity and eventual loss of pH maintenance. However, there was no effect on plasma membrane potential indicating that the effects resulting from TCDD exposure are not due to a generalized alteration of membrane fluidity or mobility but are due to interactions with specific sites within the plasma membrane and/or targets within the cell itself. These astroglial effects support epidemiologic evidence that dioxins and dioxin-like compounds may be neuroactive in the CNS.

967 TRIADIMEFON EFFECTS ON DOPAMINE TRANSPORTER SYSTEM IN THE STRIATUM AND NUCLEUS ACCUMBENS OF RAT

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Triadimefon (TDF) has been shown to be neurotoxic. In order to understand TDF mechanism of action, we investigated its effect on dopamine transporter binding sites in rat striatum (STR) and nucleus accumbens (NAC) using [^{3}H]GBR 12935, a potent inhibitor of dopamine uptake. The results obtained from the competitive binding studies revealed that in the STR, TDF competes for binding to the dopamine transporter site with a K_i of 192 ± 4 nM and an IC_{50} of 571 ±20 nM. In the nucleus accumbens, the K_i was 256 ± 42 nM and the IC_{50} = 634 ± 111 nM. Saturation studies indicated that chronic treatment with 100 mg/kg TDF for 14 days had no effect on dopamine transporter binding affinity (K_d) and receptor number (B_{max}). Data from TDF-treated rat STR revealed a K_d = 4.8 ± 0.9 nM and a B_{max} =29725 ± 5995 fmol/mg protein compared to a K_d = 3.5 ± 0.37 nM and B_{max} = 28046 ± 6331 fmol/mg protein in control group. Similar results were obtained in the nucleus accumbens of treated (K_d = 3.9 ± 1.2 nM B_{max} = 16463 ± 3423 fmol/mg protein) and control (K_d = 3.3 ± B_{max} 16106 ± 3427 fmol/mg protein) rats. Although it was concluded that TDF competes with [^{3}H]GBR 12935 for binding to dopamine transporter sites with strong affinity, TDF chronic treatment had no effect on the dopamine transporter system. Supported by NIH Grants GM0811 and RR3121

968 EFFECTS OF PRENATAL TESTOSTERONE EXPOSURE ON PROSTATE ANDROGEN SENSITIVITY OF WISTAR RATS

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Elevated circulating androgen levels during prenatal development have been implicated in the high risk for prostate cancer among African-Americans. In a rat model of prenatal testosterone exposure (pTe), we previously obtained data suggesting that pTe at a dose that reaches the fetus delays the onset of puberty in male offspring, and increases the androgen sensitivity of their prostate. To further examine this matter, Hsd:WU rats received pTe starting at day 12 of gestation by a single Silastic implant (ID 0.2 cm, OD 0.31 cm, length 3.75 cm), and were given a T-containing implant (1.6 cm) at 4 wks of age. These rats were sacrificed at day 0, 3, 7, 14, and 21 post-implantation (pi). Body weights at these time points were not different from controls, but relative weights of the dorsolateral and ventral prostate were significantly increased by 20% and 24%, respectively, at day 7 pi, and 30% (both) at day 14 pi. Relative weights of coagulating gland and seminal vesicle were increased by 35% over controls at days 7 and 14 pi. Testicular weight was significantly decreased by 18% of controls at day 21 pi. In separate experiments with rats that were not given any postnatal treatment, pTe significantly delayed: 1) the age at preputial separation from 29.3 ± 0.8 (mean ± SD) days in controls to 30.5 ± 0.8 days in pTe rats, and 2) the age at vaginal opening from 33.5 ± 1.5 days in controls to 35.2 ± 2.8 days in pTe rats. Body weights at these time points were not affected by pTe. These data support our previous findings and provide further evidence that: a) the pTe dose used reaches the fetus, b) pTe causes a delay in the onset of puberty, and c) pTe increases androgen sensitivity of male accessory sex glands. Expression of androgen-regulated prostatic secretory protein genes in rat accessory sex glands is being investigated to examine these imprinting effects of pTe at the molecular level. (supported by NIH grant CA58088)

969 PHASE-SPECIFIC DEVELOPMENTAL TOXICITY OF MONOBENZYL PHTHALATE (MBeP) IN RATS

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In our previous studies, butyl benzyl phthalate (BBP) was found to be teratogenic when administered to rats on days 7–9 and days 13–15 of pregnancy but not days 10–12. The present study was conducted to determine the phase specificity of the developmental toxicity of MBeP, which is one of the major metabolites of BBP, and to assess the role of MBeP in the developmental toxicity of BBP. Pregnant rats were given MBeP by gavage at a dose of 375, 500 or 625 mg/kg on days 7–9, days 10–12 or days 13–15 of pregnancy.

A significant increase in embryolethality was noted in pregnant rats given MBeP at 500 mg/kg and above regardless of the days of treatment. No evidence of teratogenicity was found when MBeP was given on days 10–12. A significantly increased incidence of fetuses with external malformations was found after treatment on days 13–15 at 500 mg/kg. A significantly increased incidence of fetuses with skeletal malformations was observed after treatment on days 7–9 at 625 mg/kg and on days 13–15 at 375 mg/kg and above. Deformity of the vertebral column and ribs was predominantly observed after treatment on days 7–9. Cleft palate and fusion of the sternebrae were exclusively found after treatment on days 13–15. The dependence of gestational days of treatment on the manifestation of the developmental toxicity and the spectrum of fetal malformations induced by MBeP were similar to those induced by BBP. These findings suggest that MBeP and/or its further metabolites may be responsible for the production of the developmental toxicity of BBP.

970 SAMPLING FOR TOXICOKINETIC EVALUATIONS IN CONTINUOUS INTRAVENOUS INFUSION EMBRYO-FETAL DEVELOPMENT STUDIES OF RATS AND RABBITS

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Continuous intravenous infusion in animals via an indwelling catheter is often used in preclinical safety evaluation studies to mimic the proposed clinical route and treatment regimen. The feasibility of using this route for ICH embryo-fetal development studies has been established and studies have been performed which included subsets of animals for toxicokinetic evaluations. The data generated were examined for effects upon the female and the success of sample collection.

Animals for toxicokinetics were treated in an identical manner to the main phase animals, except for sampling. Under general anesthesia medical grade silastic catheters were surgically implanted in the vena cava via the femoral vein on Day 0 of gestation for rats and 1 week prior to the insemination for rabbits. Animals were continuously infused with saline using a jacket and tether system prior to treatment and rates of 0.2 to 5 ml/kg/hr during treatment. Females were treated from Days 6/7 to Day 16/19 of gestation. Blood samples were collected from the auricular artery in the rabbits and routine sites in the rats, at multiple time points following the start of treatment, at the mid point of major organogenesis and at, and after the end of treatment. The females were given a uterine examination on day 16–19 of gestation and amniotic fluid / fetal samples were collected.

There was a high rate of successful sample collection both during infusion and after completion of treatment. These techniques did not adversely effect the maternal condition or the reproductive parameters. It was concluded that the sampling for toxicokinetic evaluations could be performed in conjunction with embryo-fetal development studies conducted via continuous intravenous infusion without modification to established protocols.

971 ACTIVE TRANSPORT OF 2-METHOXYACETIC ACID IN MOUSE WHOLE EMBRYO CULTURE

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2-Methoxyacetic acid (2-MAA), the primary metabolite and proximate developmental toxicant of 2-methoxyethanol (2-ME), accumulates in embryos and extraembryonic fluids (EAF) relative to maternal plasma at certain times of rodent gestation. Our physiologically based pharmacokinetic gestation model suggests active transport as a possible and biologically plausible explanation for this phenomenon (*TAP* 132, 103–114, 1995). This hypothesis was tested by culturing CD-1 mouse embryos (40–45 somites), with their membrane enclosures intact for 1 or 2 hr in medium that contained 5 mM 2-MAA, a level found in vivo after a teratogenic dose of 2-ME. Uptake of 2-MAA from the medium was determined by GC-MS. After 1 hr, 2-MAA levels in embryos were 8.02 ± 3.43, while those in EAF were 8.27 ± 2.20 (mean mM ± *S.D.; n* = 24). Following 30 min of preincubation with either NaCN (1 mM) or sodium azide (6.5 mM), 2-MAA levels in embryos were significantly reduced to 3.16 ± 0.46 or 2.38 ± 1.03 (n = 10 each), while those in EAF were unaffected. This regimen of treatment with potent metabolic inhibitors did not affect embryo viability, as judged by heart beat and blood circulation. Probenicid (125–1000 μM), an organic acid transport competitor, did not reduce 2-MAA accumulation into either embryos or EAF. The observations suggest that active transport of 2-MAA occurs from the medium into the embryo. The comparable degree of accumulation into EAF measured now in vitro and previously detected in vivo appears to be independent of the metabolic energy status in vitro at a time when metabolic inhibitors affect accumulation of 2-MAA by the embryo proper.

972 EVALUATING MECHANISMS OF DEVELOPMENTAL TOXICITY USING FROG EMBRYO TERATOGENSIS ASSAY - XENOPUS(FETAX)

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Mechanisms of developmental toxicology of diphenylhydantoin (DPH), trichloroethylene (TCE), and their metabolites were evaluated using Frog Embryo Teratogensis Assay - *Xenopus* (FETAX) and differently induced or inhibited exogenous metabolic activation systems (MAS). Addition of an Aroclor 1254-induced MAS detoxified DPH decreasing the potential teratogenic hazard. Cimetidine-treated MAS (cytochrome P-450 inhibited) reduced the ability of the MAS to detoxify DPH. Cyclohexene oxide-treated MAS (epoxide hydrolase-inhibited) dramatically increased the embryo lethality of DPH. Results suggest that DPH may be detoxified by cytochrome P-450. DPH and possibly a highly embryo-toxic arene oxide intermediate seem to be responsible for inducing the teratogenic effects observed in FETAX. Addition of an isoniazid-induced MAS enhanced the developmental toxicity of TCE. Addition of an Aroclor 1254-induced MAS, however, failed to alter the developmental toxicity of TCE. Cyclohexene oxide-treated MAS (epoxide hydrolase-inhibited) dramatically increased the embryolethality of TCE. Each of the metabolites tested (trichloroacetic acid, dichloroacetic acid, and trichloroethanol) were substantially less developmentally toxic on an equimolar basis than either TCE or activated TCE. Results suggested that TCE may ultimately be detoxified by cytochrome P-450. In addition, TCE and a highly embryotoxic epoxide intermediate (trichloroethylene oxide) appeared to be primarily responsible for inducing the teratogenic effects observed in FETAX. Because the Aroclor 1254-induced MAS failed to bioactivate TCE, it appeared that a pyrazole-inducible form of cytochrome P-450 mediated metabolism. Results demonstrate the utility of FETAX coupled with an exogenous MAS for evaluating toxicological mechanisms of action.

973 RETINOID Ro 23–9223 DOSIMETRY IN TERATOGENESIS

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Ro 23–9223 is a highly lipophilic aromatic retinoid with promise in preclinical models of acne, having efficacy similar to that of 13-cis-retinoic acid. To investigate the relation between Ro 23–9223 teratogenic potential, its in vivo distribution and transplacental transfer, pregnant hamsters were given oral doses of 50 or 125 mg/kg on days 8 and 9 of gestation. Gastro-intestinal bioavailability of Ro 23–9223 was low compared with 13-cis-retinoic acid and the highest levels of Ro 23–9223 were found in lung, cardiac muscle and liver, whereas only small amounts crossed the blood-brain barrier. Ro 23–9223 was transferred efficiently across the placenta; when combined with the prolonged maternal plasma half-life (10 hours), Ro 23–9223 was associated with a higher embryo:maternal exposure ratio compared to that of 13-cis-retinoic acid. Ro 23–9223 was 4-fold less potent a teratogen than 13-cis-retinoic acid based on 24 hour AUC delivered dose to the embryo. The reduced teratogenic activity of Ro 23 9223 in spite of the relative high delivered dose compared with 13-cis-retinoic acid is also shown in its 4-fold reduction in potency as measured in the in vitro limb bud micromass assay. These data demonstrate that Ro 23–9223 has an improved therapeutic ratio as compared with 13-cis-retinoic acid.

974 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY EVALUATION OF LIGHT ALKYLATE NAPHTHA DISTILLATE IN RATS

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In an on-going effort to generate toxicity data for various petroleum refinery streams, a modified OECD guideline 421 reproductive-developmental toxicity study was conducted with light alkylate naphtha (CAS # 64741–66–8) distillate vapors (LAN-D) in Sprague Dawley rats. Animals were exposed, 6 hours/day, 7 days/week, to 0, 5000, 12500, or 25000 mg/m^3 with the highest dose level exceeding 60% of the lowest explosive limit of LAN-D. Exposure began 2 weeks prior to mating and continued throughout gestation until postnatal day 4 for females or 8 consecutive weeks for males. No clinical signs

indicative of systemic toxicity were observed during the study. Inhalation exposure to LAN-D had no effect on parental food consumption, body weight, and fertility up to and including the 25000 mg/m^3 dose level. Evaluation of the reproductive organs (ovaries, testes, and epididymides) did not reveal any weight or microscopic changes attributable to LAN-D. There were no differences in the mean number of corpora lutea, implantation sites, and live pups per litter. Pups born from treated mothers showed comparable birth weights and weight gains. On postnatal day 4, all groups had a viability index ≥ 97%. No gross anomalies were observed in the pups. Under the conditions of this experiment, the no-observed-adverse-effect level (NOAEL) for LAN-D via inhalation is established at 25000 mg/m^3.
(The Petroleum Product Stewardship Committee consists of Amoco., ARCO, BP America, Chevron, Mobil, Texaco, and Unocal)

975 DEVELOPMENTAL TOXICITY OF TRITON® X-100 BY CUTANEOUS DOSING OF CD® RATS

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Timed-pregnant CD rats, 25/group, were dosed by occluded cutaneous application with 0, 12.5%, 37.5% or 100% aqueous solutions of Triton® X-100 (Octylphenol 9-mole ethoxylate; Octoxynol; CAS No. 9036-19-5) at a constant dose volume of 4 ml/kg for 6 h/d on gestation days 6–15. The pregnancy rate was 92–100%. Maternal effects (skin irritation and reductions in gestational body weight and weight gain) were observed only in the 100% group. There were no effects on gestational parameters including the number of ovarian corpora lutea, preimplantation loss, and the number of total, viable or nonviable implantations per litter or sex ratio. Also there were no effects on fetal body weight or increases in the incidences of any external malformations. In 2 fetuses of the 100% group, extra #14 thoracic centrum, arches, and ribs, with or without an extra rib #15, were noted. In addition, increased incidences of 5 skeletal variations (extra 14th rib on lumbar arch #1, rudimentary rib or bone island on cervical arch #7, unossified cervical centrum #6, poorly ossified lumbar arches, and poorly ossified or unossified sternebra #6), were observed in the 100% group. Increased incidences of supernumerary ribs (#14 on first lumbar arch) were also noted in the 12.5 and 37.5% groups, and increased incidences of fetal atelectasis were observed in the 37.5 and 100% groups. The no observed effect level (NOEL) for maternal toxicity was 1.6 g/kg/d. A NOEL for developmental effects was not established in this study.

976 MORPHOLOGICAL DEFECTS ELICITED BY 5-AZA-2'-DEOXYCYTIDINE (d-AZA) IN THE RAT - INTER-SPECIES DIFFERENCES IN RESPONSE TO d-AZA DEVELOPMENTAL TOXICITY

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5-aza-2'-deoxycytidine (d-AZA), a potent demethylating agent, causes temporally related defects in the mouse. Treatment with 1.0mg/kg on gestation day (GD) 8 resulted in axial skeletal defects; GD 9, cleft palate and vertebral defects; GD 10, hindlimb phocomelia; GD 11, digital defects. Forelimb phocomelia did not occur on any of the days treated. The current study was initiated to evaluate the embryonic response of another species, the rat, to d-AZA. Pregnant Sprague Dawley rats were treated with d-AZA or vehicle control (9% NaCl). The compound was administered via i.p. injection on GD's 9, 10, 11, or 12 (to parallel developmental staging of the mouse). The highest dose (1.0 mg/kg) elicited effects which indicated the rat's increased sensitivity to the compound as compared to the mouse. The patterns and morphological presentation of the defects also differed. GD 9 treatment was characterized by massive resorptions; GD 10, craniofacial and axial skeletal defects (most notably **missing ribs**: 12 ribs with the normal number of presacral vertebrae); GD 11, predominance of **forelimb phocomelia** and missing ribs while hindlimb phocomelia and foredigit defects were more predominant with GD 12 treatment. The analog 5-azacytidine (5-AZA) has been studied in this species and did not induce missing ribs (Rosen et al. 1990). Our data suggests there is marked inter-species differences in the temporal expression of genes involved in morphogenesis and/or the methylation patterns of these genes. Our molecular data generated in the mouse must be compared to that of the rat to further characterize the developmental dynamics responsible for our observations.

977 QUANTITATIVE ANALYSIS OF APOPTOSIS INDUCED BY 2-METHOXYETHANOL IN CD-1 MOUSE EMBRYOS ON GESTATION DAY 8

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Increased cell death in the anterior neuropore region of mouse embryos exposed to 2-methoxyethanol (2-ME) on gestation day (GD) 8 is believed to be an early event in the pathogenesis of 2-ME-induced exencephaly. We applied laser-scanning confocal microscopy (LSCM) to vital fluorochrome-stained embryos to characterize and quantify this cell death. Live embryos were exposed to either acridine orange (AO) or propidium iodide (PI) to label apoptotic cells and membrane damaged cells, respectively. Four hours after *in utero* exposure to 2-ME dosages that induce exencephaly in near-term fetuses, dose-dependent increases in AO fluorescence were quantified in the anterior neural folds [litter means = 0.2% (n = 5), 0.7% (n = 4) and 1.1% (n = 4) area for control, 250, and 325 mg/kg 2-ME groups, respectively]. In control embryos, AO fluorescence was observed at the margins of the neuroepithelium (NE) in regions where neural tube closure was ongoing. In embryos exposed to 2-ME, AO staining along the margin of the NE was expanded and also occurred in more medial regions. Negligible PI staining was observed in control and 2-ME-treated embryos, indicating that 2-ME did not damage cell membranes. Visual analysis of optical sections collected by LSCM further revealed that 2-ME-exposed embryos had abnormally shaped neural folds. The LSCM data were supported by image analysis of ApopTag™- and hematoxylin and eosin-stained histological sections. Quantitatively similar results were obtained with all three methods. This approach provides the first quantitative analysis of 2-ME-induced apoptosis in mouse embryos and demonstrates the applicability of LSCM for analysis of cell fate in intact embryos.

978 THE RELATIONSHIP BETWEEN ORGANOPHOSPHATE-INDUCED MATERNAL CHOLINESTERASE INHIBITION AND EMBRYO/FETAL EFFECTS IN THE SPRAGUE-DAWLEY RAT

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Six organophosphates (azinphos-methyl, fenamiphos, fenthion, isofenphos, oxydemeton-methyl, and tribufos) were tested for developmental toxicity, in accordance with US EPA (FIFRA) test guideline 83-3. Specifically, each organophosphate was administered by oral gavage to gravid rats on gestation days 6 through 15. Maternal cholinesterase activities (plasma; PChe, erythrocyte; RChe, and brain; BChe) were determined from a satellite group on gestation day 16. Embryo and fetal effects, including fetal brain cholinesterase activity, were assessed on gestation day 20. This report will consider the embryo/fetal effects observed at organophosphate doses that significantly inhibited maternal cholinesterase activity.

All test compounds demonstrated statistically and/or biologically significant cholinesterase inhibition at the highest dose tested. For azinphos-methyl, fenamiphos, fenthion, isofenphos, oxydemeton-methyl, and tribufos, these dose levels were 2, 3, 18, 4, 4.5, and 28 mg/kg/day, respectively. The range (mean) of inhibition, considering all compounds at the above doses, was 42–93% (72%) for erythrocyte, 37–94% (65%) for plasma, and 12–78% (54%) for brain cholinesterase activity. Despite the significant anticholinergic effects, the maternal body weight gain (gestation days 0–20) was only affected in the fenamiphos, fenthion, oxydemeton-methyl, and tribufos-treated dams. Similarly, significant clinical signs were only observed in 4/6 treatment groups. Fetal brain cholinesterase activity was not statistically significantly affected by any compound. No effects on any embryo or fetal parameters, including fetal body weight, litter size, resorptions, implantation loss, and external, visceral, and skeletal malformations, were observed following treatment with any compound. In conclusion, the administration of the aforementioned organophosphates produced biologically significant maternal cholinesterase inhibition without affecting fetal brain cholinesterase activity or any other embryo or fetal parameters. These results also suggest that cholinesterase activity inhibition is the most sensitive indicator of maternal organophosphate exposure during the conduct of a developmental toxicity study.

979 DEVELOPMENT OF A RABBIT WHOLE EMBRYO CULTURE (WEC) MORPHOLOGY SCORING SYSTEM

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Rodent WEC is a powerful tool for investigating mechanisms of normal and altered embryonic development. Although the rabbit is also a major developmental toxicity test species, there have been few attempts to culture rabbit postimplantation embryos. Consequently little is known about normal rabbit embryogenesis, let alone mechanisms of abnormal development. As a first step to advance rabbit WEC, we developed a chronological based scoring system by evaluating 18 morphological structures and 4 growth parameters of gd 9-13 *in vivo* embryos. This scoring system was then used to assess the development of gd 9 embryos grown in WEC. Embryos were cultured in 100% immediately centrifuged, heat-inactivated, serum from pregnant female rabbits, at 38°C with a continuous gas flow of 5% CO_2, 20% O_2, 75% N_2 for the first 24 hrs, and 95% O_2, 5% CO_2 for the second 24 hrs (Ninomiya *et al.*, Toxicol. *In Vitro* 8:109, 1993). After 48 hrs of culture, *in vitro* growth was comparable to gd 11 *in vivo* growth: *in vitro* somite number was 94.6% of *in vivo* (35 ± 3.96 vs. 37 ± 1.36, respectively); *in vitro* head length (mm) was 79.3% of *in vivo* head length (2.15 ± 0.28 vs. 2.71 ± 0.31, respectively); *in vitro* crown-rump length (mm) was 76.2% of *in vivo* length (4.42 ± 0.36 vs. 5.8 ± 0.52, respectively), and *in vitro* protein concentration per embryo (μg–) was 78.7% of *in vivo* (531.6 ± 131.44 vs. 675.2 ± 157.8, respectively). These results demonstrate that rabbit postimplantation embryos can be cultured *in vitro* and also provide a system for quantitative evaluation of embryo morphology. The further development of rabbit WEC represents an important step toward addressing critical data gaps in this species.

980 DEVELOPMENTAL TOXICITY TESTING OF ALTERNATIVE VEHICLES: PEG 400, CREMOPHOR AND CARBOXY-METHYLCELLULOSE: COMPARISONS WITH METHYLCELLULOSE

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To test the toxicity of a potentially new drug candidate, the compound has to be formulated in a suitable vehicle. When presented with compounds with low aqueous solubility and poor bioavailibility or lack of proper parenteral formulation, alternative vehicles must be sought out. However, the developmental toxicity of these novel vehicles is generally unknown and must be assessed before they can be accepted as vehicles.

Timed pregnant female Sprague-Dawley rats were randomly assigned to 4 dose groups (10/group). Animals were dosed between gestational days 6–17 by oral gavage, with 0.5% methylcellulose, PEG 400, cremophor, and 0.1% carboxy methylcellulose. Body weights and food consumptions were recorded daily and clinical signs were checked twice daily.

Cesarean sections were performed on gestational day 21. Each uterus was weighed prior to removal of the fetuses. The fetuses and their placentas were weighed individually and examined for external anomalies. The numbers of corpora lutea from each ovary, implantation sites, resorptions and the number of viable and dead fetuses were recorded for each dam. On one-half of the fetuses, visceral exams were performed on the day of the cesarean section and the remaining fetuses were processed for skeletal examination.

No treatment related clinical signs were seen in any group. There were no vehicle related mortalities. There were no treatment related differences in body weights, food consumption and reproductive parameters between the groups. There were no biologically significant differences between the dose groups in terms of external and visceral anomalies. Observations from both visceral and skeletal examinations will be presented. The data from this study provide some assurance for the use of alternate vehicles in the safety testing of novel compounds.

981 DEVELOPMENTAL TOXICITY STUDY OF BENOMYL IN RABBITS

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The developmental toxicity of benomyl, the primary component of the foliar fungicide Benlate®, was assessed in rabbits by orally administering suspensions of the test material in 0.5% methyl cellulose from around the time of implantation to the end of gestation (Days 7–28 of gestation). Groups of 20 time-mated rabbits were gavaged at daily dose levels of 0, 15, 30, 90, or 180 mg/kg. The does were euthanized on Day 29 of gestation and the offspring were weighed and examined for external, visceral, and skeletal alterations. Maternal toxicity was demonstrated at 180 mg/kg/day as decreased food consumption. The significant, dose-related reductions in mean maternal food consumption occurred primarily during the first and second weeks of dosing. In addition, there was a significant increase in the number of does at 180 mg/kg that had stained tails. Developmental toxicity was demonstrated at 180 mg/kg/day. This was manifest as a slight, but statistically significant, increase in the incidence of small renal papillae. There was no evidence of either maternal or developmental toxicity at 90 mg/kg or lower. Thus, the maternal and developmental no-observed-effect level (NOEL) was 90 mg/kg. Therefore, under these experimental conditions, benomyl was not uniquely hazardous to the rabbit conceptus.

982 REPRODUCTIVE PERFORMANCE ALTERED BY CHEMICAL MIXTURE

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Leaching of chemicals from hazardous waste sites has resulted in contamination of ground water with complex mixtures of chemicals. Effects of exposure of meat-type breeder chickens to a chemical mixture in drinking water and restricted (R) water from 27 through 38 weeks-of-age were evaluated. Treatment groups included (1) control water; (2) low levels of chemical mixture (arsenic, benzene, cadmium, lead and trichloroethylene; .9, 1.25, 5.1, 7 and .65 ppm, respectively) in drinking water; (3) high level of chemical mixture (10 times of low), (4) low/R and (5) high/R (consumption of control water restricted to volume consumed by chickens receiving low and high levels of chemical mixture). Water consumption was significantly decreased by the high level of chemical mixture, however, feed consumption was not altered by any of the treatments. Increasing levels of chemical mixture in drinking water resulted in significantly decreased hen body weight, egg production, egg weight, hatchability and significantly increased embryonic mortality. Adverse effects of low levels of chemical mixture on egg hatchability and embryonic mortality were due to chemicals, not restricted water consumption.

983 TOXIC EFFECTS OF CHINESE MEDICINAL HERBS ON PREGNANT MICE

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Listed in the Chinese Pharmacopoeia are around 60 medicinal herbs which pregnant women are advised against taking or to take with great caution. After the toxic herbs, minerals and infrequently used herbs are excluded, there are about ten to twenty frequently used herbs which may exert adverse effects on pregnant women. Through a collaboration with the National Research Institute of Chinese Medicine, Taiwan, we have evaluated the reproductive toxicity of 10 selected herbs. The aqueous extracts of the whole plants were prepared and provided orally to pregnant mice for 4 consecutive days before implantation. Of the 10 herbs tested, *Rheum offinicale* was the most toxic which caused severe uterine damage and a dose-dependent increase of the percentage of abortion in the mice. The levels of intra-uterine interleukin-6 and serum progesterone measured by ELIZA method and radioimmunoassay were significantly decreased and correlated with the abortion rates.

984 COMPARATIVE STUDIES OF THE ACUTE TOXICITY OF MTBE IN JAPANESE MEDAKA AND CULTURED RAT BRAIN MICROVESSELS

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Methyl-tert butyl ether (MTBE) is used in the oxygenation of fuels at concentrations of up to 12%. Workers in the petroleum industry with blood levels of 1.80 ug/ml reported a high incidence of neurologic symptoms, including headaches, dizziness, and nausea. While these symptoms are suggestive of vascular involvement, little information is available on the effects of MTBE on cerebral microvasculature. Studies examining the effects of MTBE at concentrations up to 0.01–0.3% in the Japanese medaka (*Oryzias latipes*)

developing embryo resulted in a unique lesion of the developing embryonic vasculature. The embryonic vasculature failed to develop, while other non-vascular tissues developed normally until vascularization became essential for further development. Studies were undertaken to determine if MTBE would alter rat brain microvessels *in vitro*. Rat brain endothelial cells were isolated by centrifugation on dextrose and percoll density gradients and maintained in culture. Isolated rat brain endothelial cells grown on Matrigel® elaborate cytoplasmic processes which, after two days in culture, form micro-capillaries. Exposure of cultured rat brain endothelial cells to MTBE (0.03–3.0 g/L) resulted in a dose dependent reduction of microcapillary formation. The higher concentrations (0.3–3.0 g/L), produced necrosis and reduced cell numbers. These data suggest that microcapillaries may be a primary target of MTBE and that toxicity may be mediated by endothelial cells *in vivo*. (Supported by PHS-NIH ES 06103, ESO502 and NJAES 01407).

985 DEVELOPMENTAL TOXICITY STUDY IN RATS AND RABBITS ADMINISTERED A 20% LIPID EMULSION CONTAINING A 3:1 RATIO OF MEDIUM CHAIN TRIGLYCERIDES:LONG CHAIN TRIGLYCERIDES

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A 20% lipid emulsion containing a 3:1 ratio of medium chain triglycerides:long chain triglycerides (3MCT:1LCT) was intravenously administered to rats and rabbits at dosages of 1 and 4.28 g lipid/kg body weight at dose volumes of 5 and 21.4 mL/kg, respectively, once daily during organogenesis to assess the potential developmental toxicity of the test article. The control group received 0.9% saline at a dose volume of 21.4 mL/kg. Animals were observed for clinical signs of toxicity and adverse effects on body weight and feed consumption. On Day 20 (rat) or 29 (rabbit), females were euthanized and examined for maternal and embryo/fetal toxicity. Fetuses were removed, weighed, and examined for external, soft tissue and skeletal abnormalities. Dosages of 4.28 g lipid/kg resulted in lower feed consumption for rats and rabbits, an expected finding based on the high-caloric nature of the test article. Potentially test article-related gross necropsy findings for rats given 4.28 g lipid/kg were present at a low incidence. There were no adverse effects on fetal parameters for rats even in the presence of some maternal toxicity. However, embryo and fetal toxicity and teratogenicity were present for rabbits given 4.28 g lipid/kg. The fetal effects in the rabbit were probably the result of dietary deprivation, maternal toxicity, or both rather than a direct teratogenic effect. Under the conditions of this study, the NOEL level for developmental toxicity was greater than 4.28 g lipid/kg for rats and was greater than or equal to 1 g lipid/kg but less than 4.28 g lipid/kg for rabbits.

986 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY TEST OF DERMALLY ADMINISTERED HYDRODESULFURIZED KEROSINE IN RATS

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In an ongoing program to generate toxicity data for refinery streams, a modified OECD Guideline 421 toxicity study was conducted with hydrodesulfurized kerosine (HDS-K, CAS#64742-81-0) in Sprague-Dawley rats. HDS-K diluted in a mineral oil (MO) vehicle was tested in an acute dermal irritation assay to identify minimally irritating doses. The dermal penetration potential of HDS-K neat and in MO was established in an in vitro percutaneous absorption study. HDS-K diluted to 494 (60%), 330 (40%), and 165 (20%) mg/kg/day in Squibb MO was applied daily at 1 ml/kg to the shaved backs of rats for seven weeks (pre-mating, mating to day 19 of gestation) to females and 8 weeks to males. Dams and litters were sacrificed on postpartum day 4; males sacrificed shortly thereafter. HDS-K produced slight to moderate skin irritation at the highest dose in both sexes but no apparent maternal, reproductive or developmental toxicity. No clinical signs of toxicity, and no effect on body weight, food consumption, fertility or absolute organ weights were observed. However, relative kidney weights were heavier in male rats at the high dose. There were no HDS-K related microscopic changes in testes, epididymides or ovaries of parental animals but skin changes were observed in male rats in all groups and in females at the high dose. There were no differences in the mean number of corpora lutea, implantation sites and live pups per litter. Pups born from treated dams showed comparable body weights and weight gains. Viability Index on postpartum day 4 was >97%. No gross anomalies were observed in pups. Under conditions of this study, the NOAEL for HDS kerosine is 494 mg/kg/day for reproductive/developmental toxicity in rats.

1 is Amoco, ARCO, BP America, Chevron, Mobil, Texaco, Unocal

987 EVALUATION OF THE FROG EMBRYO TERATOGENESIS ASSAY-XENOPUS (FETAX) AS A SCREEN FOR DEVELOPMENTAL TOXICANTS

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FETAX is a 96 hour static renewal test, designed to detect developmental toxicants, using the embyros of the frog *Xenopus laevis*. Primary endpoints include mortality, malformation and growth inhibition. FETAX offers several advantages in hazard assessment including time and cost-effectiveness, technical ease, and versatility. A number of validation studies using compounds of known mammalian developmental toxicity have suggested that FETAX will have a predictive accuracy of greater than 90%. In the present study we evaluated the utility of FETAX to identify the potential developmental toxicity of a group of diverse chemicals. A total of 16 chemicals, 6 of which have been shown to be teratogenic *in vivo*, 7 of which were embryotoxic (but not teratogenic) *in vivo*, and 3 which did not produce any developmental toxicity *in vivo* were evaluated in this assay. Results of the FETAX test with these 16 blind-coded compounds correctly predicted that 6 chemicals had strong teratogenic potential, 7 had low to moderate hazard potential and 1 posed little if any hazard. The remaining 2 compounds, which were not developmentally toxic *in vivo* were considered to possess a low to moderate hazard potential. In addition, within a family of chemistry, analogs could be ranked according to potency. In summary, these results confirm that the FETAX assay is predictive and can be useful in an integrated biological hazard assessment in the preliminary screening of chemicals.

988 REPEATED EXPOSURE OF RATS TO NP-439 DOES NOT PRODUCE DEVELOPMENTAL TOXICITY OR NEUROTOXICITY

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NP-439, a pentaerythritol ester with mixed acids, is intended for use as a lubricant base stock. In order to verify the safety of NP-439, developmental toxicity and 28-day oral toxicity with functional observational battery (FOB) studies were conducted in rats. In the developmental toxicity study, pregnant female Sprague-Dawley rats were dosed with 100, 500, or 1000 mg/kg/day of NP-439 on gestation day (GD) 6 – 15. No maternal toxicity or embryotoxicity were seen at any dose level. NP-439 did not produce increases in malformations or variations at any dose level. In the 28-day study, male and female Sprague-Dawley rats were dosed with 100, 500, or 1000 mg/kg/day of NP-439, evaluated in the FOB on days 8 and 27, and examined histopathologically. No treatment-related effects were seen in any dose group for all parameters measured. The no-observed-adverse-effect-level (NOAEL) for both developmental toxicity and subchronic/neurotoxicity was 1000 mg/kg/day.

989 DEVELOPMENTAL TOXICITY STUDY IN RATS WITH A NOVEL CYTOKINE REGULATING AGENT (CRA™), HP 228

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HP 228, a heptapeptide, has been shown to be an effective Cytokine Regulating Agent (CRA®) in a variety of *in vivo* models, including endotoxemia, pain, inflammation, ischemia-reperfusion, chemotherapeutic toxicity, and diabetes. This study was conducted to determine if HP 228 had any developmental toxic effects. HP 228 was administered subcutaneously, every 8 hours, to six groups (n = 10) of timed pregnant Sprague-Dawley rats at doses of 0, 5, 50, 500, 5000, or 15000 μg/kg on gestation days (GD) 6–16. In comparison, the efficacious dose of HP 228 ranges from 5–500 μg/kg. Maternal toxicity was not seen at any dose of HP 228. Gravid uterine weight was unaffected. There were no treatment-related effects on the number of corpora lutea, implantations, resorptions, live fetuses, sex and live/dead ratios, and external abnormalities. Mean fetal body weights were significantly ($p<0.05$) lower in the 15000 μg/kg HP 228 group. However, this may be due to the fact that the high dose group included two large litters in which all the fetuses were equally small. When fetal body weights were normalized by litter size,

there were no significant differences between groups. These results indicate that at doses as high as 15000 μg/kg, HP 228 does not induce any significant developmental toxic effects.

990 AMINOCARB EMBRYOTOXICITY IN RAT CONCEPTUSES: EXPOSURE IN WHOLE EMBRYO CULTURE AND BY DIRECT INTRAAMNIOTIC MICROINJECTION

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The carbamate pesticide aminocarb can be co-oxidized during arachidonic acid metabolism by prostaglandin H synthase, producing formaldehyde, an *in vitro* embryotoxicant. Aminocarb can also be degraded nonenzymatically to glutathione (GSH)-depleting benzoquinones. Evidence for a role of co-oxidation and altered GSH status in the mechanism of other embryotoxicants suggests that aminocarb may be a useful model compound for the study of these pathways. Gestational day 10 rat conceptuses were treated with aminocarb (150, 250, 300, 500 and 600μM) in whole embryo culture medium for 24 hr, resulting in a dose-dependent increase in mortality, frequency of malformations and in decreased growth parameters. Protein and DNA contents of embryo and visceral yolk sac (VYS) decreased as aminocarb concentrations increased. Aminocarb exposures greater than 150 μM produced pooled blood in the cranial region and especially around the periphery of the optic cup. Hypoplasia of the prosencephalon was present at every dose and increased in a dose-dependent manner. Abnormal axial rotation was apparent only at or above doses of 500 μM. HPLC analysis of whole conceptuses exposed to aminocarb for 24 hr revealed significant decreases in cysteine levels beginning with 300 μM and significant decreases in GSH following 500 μM aminocarb treatment. When tissues of whole conceptuses exposed to 500 μM aminocarb for 24 hr were assessed separately, GSH concentrations were decreased by 20% in embryos and 40% in VYSs. Microinjection experiments suggest that embryotoxic effects of aminocarb might be mediated by biotransformative processes occurring in the VYS. A dose of 100 ng/μl aminocarb (500 μM) added directly to culture media resulted in 70% incidence of blood pooling and 85% incidence of prosencephalic hypoplasia after 24 hr. However, microinjection of 2,000 ng/μl aminocarb into the amniotic sac produced no blood pooling or prosencephalic hypoplasia. Research supported by NIH grants ESO5235 and ESO7062 and EPA grant CR 821964.

991 DIFFERENTIAL *IN VITRO* TOXICITY OF MOUSE AND RAT EMBRYOS FOLLOWING DIRECT INTRA-AMNIOTIC EXPOSURE TO METHANOL, FORM-ALDEHYDE AND SODIUM FORMATE

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Exposure of pregnant rodents to methanol produces a greater incidence and severity of malformations and toxicity in mouse vs rat conceptuses, irregardless of whether exposure occurred in vivo or in vitro. The mechanistic basis for differences of greater sensitivity in the mouse is unknown, although we have obtained evidence for a possible role of the visceral yolk sac (VYS) in mediating the observed embryotoxicity through differences in biotransformation capacities. Intraamniotic microinjection of methanol or its metabolites formaldehyde and formic acid provides a means whereby the VYS can be bypassed and direct chemical effects on the embryo evaluated. Methanol microinjection in 4–10 somite CD-1 mouse embryos resulted in a 75% decrease in viability at doses as low as 45 μg, whereas amounts in excess of 178 μg were required at the same stage to produce an equivalent effect in the rat. Four-fold lower amounts (0.26–0.50 and 1.51–2.00 μg in mouse and rat, respectively) of formaldehyde were required in the mouse to produce a 76% decrease in viability, a 50% incidence of incomplete neural tube closure and a significant increase in axial rotation defects. Intraamniotic exposure to sodium formate resulted in negligible differences between species for any parameter measured. These studies demonstrate that differences in mouse sensitivity to methanol may be due to direct effects in the embryo proper and are likely not mediated through the VYS. This work was supported by EPA grant CR821964.

992 RETINOIC ACID ALTERS DISTRIBUTION OF GLYCOCONJUGATES IN THE DEVELOPING SECONDARY PALATE

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In mice, retinoic acid (RA) given on gestation day (GD) 12 results in delayed palatal shelf elevation and subsequent cleft formation. The present study was undertaken to determine whether RA alters the pattern of glycoconjugate expression during secondary palate development. On GD 12, gravid CD-1 females were given an oral dose of RA or vehicle. Embryos were collected at 10, 16, 28, 40, and 52 hours post exposure. Coronal sections were stained with 4 lectins (PNA, SNA, RCA-1, WGA). Differences in lectin staining were compared as plus or minus, and by using computer assisted image analysis. The imaging data were analyzed using the Mann-Whitney test. RA decreased PNA (galactosyl (β-1,3) N-acetylgalactosamine linked glycoconjugates) staining of the palatal mesenchyme 2 fold and 2.7 fold at 10h and 16h, respectively. Significant differences were not observed at 28h. RA treatment resulted in decreased extracellular SNA (alpha 2,6 and alpha 2,3 terminal galactose linked glycoconjugate) and RCA-1 (terminal galactose and N-acetylgalactosamine linked glycoconjugates) staining in the oral and nasal mesenchyme through 28h post exposure. Staining with WGA (N-acetylglucosamine and sialic acid linked glycoconjugates) was unchanged at the time points examined. RA did not affect the epithelial staining patterns of any of the lectins we investigated. These results suggest that the RA-induced delay in shelf elevation may be partially mediated through altered expression of glycoconjugates in the extracellular matrix of palatal mesenchyme.

993 INVESTIGATION OF DEVELOPMENTAL NEUROTOXIC EFFECTS OF DIET RESTRICTION

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The effects of dietary restriction in groups of 30 rats during pregnancy and lactation on the post natal development of the resulting offspring was investigated. In addition, the effects of daily gavage dosing, the normal route of administration for studies of this type, was explored.

Dietary restriction, either by feeding a fixed amount of diet (approximately 80% of ad libitum food consumption) or by controlling the time of feeding (ad libitum diet for 6 hours per day), resulted in lower maternal bodyweights but had no effect on reproductive performance. Bodyweight of the resultant pups was similar to control at birth and on day 5 but on day 12 was about 17% lower than control and remained lower than control to day 63. Despite the effects on early post-natal growth there were no effects on detailed clinical observations, the age of vaginal opening or preputial separation, motor activity, learning and memory, startle response or on brain weight or pathology.

Daily gavage doses of sterile water (10ml/kg) to rats during pregnancy and lactation (GD7-LD11) had no adverse effects in terms of clinical signs, bodyweight, food consumption or reproductive performance and resultant offspring were indistinguishable from offspring of untreated control rats.

994 AN ASSESSMENT OF THE DEVELOPMENTAL TOXICITY OF TRIETHYLENE GLYCOL (TEG) AND TRIETHYLENE GLYCOL DIMETHYL ETHER (TEGDE) IN DROSOPHILA MELANOGASTER

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As part of an ongoing effort to assess the developmental toxicity of selected glycol ethers and their metabolites, *Drosophila* were exposed throughout development (egg through third instar larva) in culture vials to medium containing equimolar concentrations (.0013–1.64mM/vial) of triethylene glycol (TEG) and triethylene glycol dimethyl ether (TEGDE). Each vial contained 1g of powered medium and 5ml of distilled deionized water or a solution of test chemical in water. A mated, untreated, Oregon-R wild-type female was added to each culture 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; a morphological defect shown to occur with an increased incidence in flies exposed to developmental toxicants. The incidence of bent bristles was statistically increased ($p<0.001$) compared to concurrent controls (chi-square) at the two highest TEG concentrations (38/181 at 1.33mM; 29/99 at 1.64mM). For TEGDE, the incidence of bent bristles was increased at 0.34mM (10/159; $p<0.01$) and at 0.66mM (28/171; $p<0.001$) and 0.99mM (6/25; $p<0.001$) - no flies emerged at 1.33

and 1.64mM. In contrast to these results, treatment with the documented developmental toxicant 2-methoxyethanol (2ME) resulted in statistically significant increases in bent humeral bristles at concentrations as low as 0.085mM in experiments reported previously using a similar protocol. The results with TEG and TEGDE parallel the developmental toxicity seen in mammals and support further utilization of this *Drosophila*-based assay as a prescreen for developmental toxicants.

995 LACK OF DEVELOPMENTAL TOXICITY IN RATS TREATED WITH EXXAL 8N ALCOHOL

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Exxal 8N (CAS #91994-92-2) is a branched C_8 alcohol. This study was conducted to provide information on the developmental toxicity of Exxal 8N alcohol in the rat, according to the European Union Annex V Methods for Determination of Toxicity, "Teratogenicity". Based on results from a range-finding study, Exxal 8N was administered by oral gavage to 4 groups of CRL:CDBR mated female rats at doses of 0, 100, 500, and 1000 mg/kg/day on gestation days (GD) 6–15. Signs of maternal toxicity were apparent at the 1000 mg/kg/day dose level, as indicated by adverse clinical signs and reductions in body weight gain and food consumption. Fetal toxicity and malformations were not observed. Increases in fetal skeletal variations were seen in mid and high dose groups when compared to controls. However, these incidences were regarded as alternative normal patterns of development. Therefore, the maternal no-observable-adverse-effect-level (NOAEL) for Exxal 8N was established at 500 mg/kg/day, and the fetal NOAEL was established at 1000 mg/kg/day. This study demonstrates that Exxal 8N was not a selective developmental toxicant and was not embryotoxic under conditions of this study.

996 METALLOTHIONEIN PRE-INDUCTION BY ZINC AND ISOTRETINOIN TERATOGENICITY IN CD-1 MICE

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Metallothionein (MT) induction by modulation of zinc (Zn) has been shown to offer protection against subsequent free radical (FR) exposure. This investigation evaluated the potential protective role of MT against the teratogenic effects of isotretinoin (ITR), a dermatological drug which causes FR damage, in CD-1 mouse embryo. Significant induction of MT by Zn supplementation was observed in mouse embryo both *in vivo* and *in vitro*. On gestational day (GD) 6.5, pregnant mice received a subcutaneous Zn injection of 0 (saline), 20 and 40 mg/kg resulting in embryonic MT concentrations of 22.0, 57.6 and 81.9 μg/g protein, respectively, after 48 hrs. Embryos were extracted at GD 8.5 and incubated at 0 and 15 μM Zn for 48 hrs. There was a sixfold increase in MT expression in the embryos of the Zn treatment group. Pre-induction of MT by Zn exposure was found to offer protection against subsequent exposure to ITR. Embryos (GD 8.5) were extracted and cultured for 24h with 15 μM Zn or with saline, followed by a 24h ITR or saline exposure. After the culture period, embryos were examined for growth and development. Growth parameters were similar in all four experimental groups (Zn-saline, saline-saline, Zn-ITR and saline-ITR). Observed embryonic malformations included abnormal brain, forelimb and flexion, blebs and enlarged pericardium. There was a significant reduction in the frequencies of abnormal brain and flexion in the Zn-ITR group (0 and 10.5% respectively) when compared to the saline-ITR group (20 and 40% respectively). The four experimental groups showed similar frequencies of abnormal forelimb and enlarged pericardium. Bleb frequency was significantly higher in the Zn-ITR group than in the saline-ITR group (36.6 and 5%, respectively). Overall, frequencies of abnormal embryos were 27.8% for the Zn-saline group, 38.9% for the saline-saline group, 36.8% for the Zn-ITR one and 60% for the saline-ITR group. These results suggest that modulation of MT levels in mouse embryo by Zn can protect FR toxicity of teratogen such as ITR. (Supported by grants by NSERC of Canada)

997 ENHANCED PHENYTOIN EMBRYOPATHY IN p53-DEFICIENT MICE: CHARACTERISATION OF EMBRYONIC p53 GENOTYPE AND THE p53, p21 AND Bax DNA DAMAGE RESPONSE PROTEINS

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Phenytoin damages embryonic DNA, the repair of which is facilitated by the p53 tumor suppressor gene. To directly determine the relevance of an embryonic DNA damage response, teratogenesis and p53, p21 (growth arrest) and bax (apoptosis) proteins were assessed in p53-normal and p53-deficient mice treated on gestational days 12 and 13 with phenytoin, 65 mg/kg ip. In utero death (fetal resorptions) and postpartum lethality both were increased 4-fold in heterozygous (+/-) p53-deficient dams compared to p53-normal (+/+) controls ($p<0.001$). Within +/- p53-deficient dams, +/- and homozygous (-/-) p53-deficient embryos were respectively 11 and 14 times more likely to die in utero than their p53-normal littermates ($p<0.005$). Also, -/- p53-deficient fetuses were twice as likely to die postpartum than either their +/- p53-deficient or +/+ p53-normal littermates ($p=0.065$). Maternal p53 affected embryonic susceptibility, in that all embryonic genotypes from p53-normal dams were resistant to phenytoin embryopathy. Within +/-p53-deficient dams, -/- p53-deficient fetuses exhibited more delay in phalangeal ossification than their +/- and +/+ littermates, but not from offspring of +/+ p53-normal dams. In +/+ embryos on GD 13, p53 protein was maximally induced 26 hr after phenytoin treatment, while p21 and bax showed a complex response and intra-litter variation. These results constitute the first evidence for an embryonic DNA damage response, and indicate a teratological suppressor role for p53 in phenytoin teratogenicity. (Support: Medical Research Council of Canada; Hospital for Sick Children Foundation)

998 ASSESSMENT OF LATHYROGENIC ACTION OF AMINOETHYL NITRATE (AEN) IN RAT FETUSES

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Lathyrogenic agents are known to produce fetal anomalies which include cleft palate, and malformationsof lims and vertebral column. A teratogenicity study of AEN was performed to examine the lathrogenic action on rat fetuses. AEN was administered orally to Sprague-Dawley rats on days 13 to 15 of pregnancy at doses of 0, 150 or 500 mg/kg. Other rats recieved AEN in the same manner on day 15 or 16 of pregnancy at a dose of 1000 mg/kg. Pregnant females were sacrificed on day 20 of pregnancy and their fetuses were examined morphologically. An increase in the frequency of cleft palate and bony curvature was observed in 500 mg/kg treated on days 13 to 15 and 1000 mg/kg treated on day 15 of pregnancy. Organ culture was performed to determine a direct action of AEN on the palatal shelves and fore limbs. Female rats were sacrificed on day 15 of pregnancy, and the fetuses were removed from the uterus and placed in Tyrode's solution. Palatal shelves and fore limbs were dissected and cultivated separately in a 25 ml bottle with 0, 150, 300 μ g/ml AEN in 2 ml of BGJ medium at 37°C in an atmosphere of 50%O_2, 5%CO_2 and 45% N_2 for 48h. At the end of the culture period, the palatal shelves were examined for the degree of fusion at the midpalate region. The fore limbs were fixed and stained with alcian blue for skeletal examination. The fusion of the palatal shelves was significantly inhibited and skeletal malformations of fore limbs were observed in the 300 μ g/ml AEN treated group. From our results, AEN has lathyrogenic action in the fetuses of rats and it may directly affect the palatal shelves and fore limbs.

999 RETINOIC ACID-INDUCED PERTURBATIONS IN ENDOCHONDRAL BONE FORMATION IN MOUSE EMBRYOS

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Retinoic acid (RA) is well recognized both as an endogenous morphogen and a teratogen in humans and laboratory animals. To study mechanisms involved in RA-induced limb defects, pregnant CD-1 mice were gavaged with 70 or 90 mg RA/kg on gestation day (GD) 12. Embryos were collected at GD 13, 14, 15 and 17 for histopathology. Embryos were also collected at GD 17 for clearing and alizarin red staining. Long bones of all RA-exposed embryos were shorter than in controls, with a mid-diaphyseal bend. Changes were most pronounced in high-dose embryos, with rough, irregular bone surface and more prominent bending of the diaphysis. There were decreased numbers of digits and fewer ossification sites in both front and rear paws of RA-exposed embryos. Histologically, at GD 13, there was a dose related delay in cartilage hypertrophy, with collapse of the hypertrophic zone. Hypertrophic zones of cartilage were present at GD 14 and 15 but had a central region of compressed chondrocytes. Ossification of long bones lagged behind control embryos at all time points. These results suggest that RA-induced limb defects in CD-1 mice exposed on GD 12 are due to an inhibition of cartilage maturation and bone formation.

1000 FERTILITY, PERI/POSTNATAL AND DEVELOPMENTAL TOXICITY STUDIES IN SPRAGUE-DAWLEY (CD) RATS WITH AL-2178

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AL-2178 is a corticosteroid, indicated for postsurgical inflammation and uveitis when formulated in an ophthalmic suspension. Fertility and peri/postnatal toxicity were evaluated at oral gavage doses of 50, 150 and 500 mg/kg/day, and developmental toxicity at 100, 500 and 1000 mg/kg/day (in 0.5% carboxymethylcellulose). In the fertility study, males were treated beginning 4 weeks before mating and females were treated for 2 weeks before mating through gestation day (gd) 6. Females were necropsied on gd 15 and males were necropsied after 10 weeks of exposure. Reproductive parameters were evaluated. In males, there were dose-related decreases in body weight (bw), bw gain and food consumption (fc) in all groups; small adrenals in the 150 and 500 mg/kg groups; calculi in the urinary system and decreases in accessory sex organ weight relative to brain weight in the 500 mg/kg group. In females, decreases in bw and bw gain occurred in the 500 mg/kg group, and decreases in gestation fc and increases in early resorptions occurred in the 150 and 500 mg/kg groups. In the peri/postnatal toxicity study, females were treated from gd 6 through lactation day 20 and necropsied. F1 developmental and behavioral parameters were evaluated. Selected F1 were mated at 12 weeks and allowed to deliver. Animals were necropsied following lactation. Decreases in F0 bw and bw gain occurred during gestation and lactation, and decreases in F1 bw occurred during lactation and the growth phase in the 500 mg/kg group. In the developmental toxicity study, females were treated on gd 6–17, necropsied on gd 20 and fetuses were evaluated. Decreases in bw and bw gain occurred in females in the 500 and 1000 mg/kg groups. The no-observed-effect level (NOEL) was below 50 mg/kg/day for males and 50 mg/kg/day for females in the fertility study; 150 mg/kg/day for F0 females in the peri/postnatal study; and 100 mg/kg/day for females and 1000 mg/kg/day for fetuses in the developmental toxicity study.

1001 NOSE-ONLY INHALATION DEVELOPMENTAL TOXICITY STUDY OF A SALBUTAMOL SULFATE/HFA-134a METERED DOSE INHALER IN RATS

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The proposed replacement of chlorofluorocarbons (CFCs) with non-ozone depleting propellants such as HFA-13a necessitated additional toxicity testing of reformulated metered dose inhalers. Of particular concern is the design and conduct of nose-only developmental toxicity studies specifically relating to the stress of confinement of dams and its effect on the study. Accordingly, the potential developmental toxicity of Airomir™TM (salbutamol sulfate/HFA-134a formulation) delivered via metered dose inhalers was evaluated. Three doses of salbutamol sulfate (0.4, 2.8, and 10 mg/kg/day and placebo formulations were administered as aerosols by nose-only inhalation to groups of 23 timed-pregnant primiparous Sprague-Dawley rats via metered dose inhalers. Concurrent control groups of equal size were exposed to filtered air only and served as a sham control. The dams were exposed 1 hour/day on gestation days 6 through 15. Prior to mating, the rats were acclimated to restraint in the nose-only tubes. Maternal body weights, body weight gain, uterine weights, and fetal evaluations were performed. In addition, core body temperatures were determined prior to and immediately after exposure. Salbutamol and its placebo formulation resulted in transient body temperature depression and overt transient clinical signs in the dams with no other significant toxic or teratogenic effects in the dams or fetuses. The results showed that the Airomir™TM formulation given at the maximum feasible inhalation concentrations did not cause teratogenic effects. These studies also confirmed that nose-only developmental toxicity studies can be successfully conducted with proper handling and acclimation to nose-only tubes.

1002 DEVELOPMENTAL TOXICITY STUDY OF MANGAFODIPIR TRISODIUM IN NEW ZEALAND WHITE RABBITS

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Mangafodipir trisodium (MnDPDP) is an intravenously administered manganese chelate undergoing clinical evaluation for magnetic resonance imaging contrast enhancement of the hepatobiliary system. The anticipated single clinical dose for adults is 5 μmol/kg body weight. MnDPDP, as well as the inorganic salt, MnC12, were previously shown to induce a specific syndrome of fetal skeletal abnormalities in rats. The syndrome malformations included angulated or irregularly shaped clavicle, femur, fibula, humerus, ilium, radius, scapula, tibia or ulna. The objective of the present study was to assess the developmental toxicity of MnDPDP in a second mammalian species, the New Zealand White rabbit. MnDPDP was intravenously administered daily to groups of rabbits (22 per group) on Days 6 through 18 of pregnancy at doses of 0 (saline), 5, 20, 40 and 60 μmol/kg MnDPDP. Fetuses were examined on Day 29 of pregnancy for external, visceral, and skeletal abnormalities. Treatment with MnDPDP did not result in overt symptoms of toxicity, and there were no significant effects on body weight gains or feed consumption. The maternal NOAEL, therefore, was 60 μmol/kg MnDPDP. Treatment with MnDPDP resulted in a significant increase in postimplantation loss at 60 μmol/kg, but there was no significant increase in external, visceral or skeletal abnormalities at any dose. The developmental NOAEL for MnDPDP, therefore, was 40 μmol/kg. These results indicate that the developmental toxicity profile of MnDPDP differs considerably in the rat and rabbit. In the rat, this compound induces specific skeletal abnormalities whereas in the rabbit, embryo/fetal toxicity is the most sensitive developmental endpoint with no evidence for the induction of specific skeletal abnormalities.

1003 RETINOL PALMITATE (RP) AS A POSITIVE CONTROL AGENT IN RAT AND RABBIT DEVELOPMENTAL TOXICITY STUDIES

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A data-base for the use of RP as an oral positive control agent was developed in CD® rats and New Zealand White rabbits. RP was used as a positive control agent in three developmental toxicity studies in each species. Based on dose range-finding studies, doses on GD9 and 10 were 1000 and 300 mg/kg/day (i.e., 250,000 and 75,000 IU/kg/day) in rats and rabbits, respectively. Maternal signs, body weights and food consumption were evaluated. Maternal necropsy and cesarean section evaluations were performed on GD20 and 29 in rats and rabbits, respectively. All viable fetuses were weighed, sexed and externally examined. All rabbit fetuses were freshly examined for visceral malformations and variations (Staples' technique) followed by skeletal examination. In rat studies, 50% of the fetuses underwent visceral examination (Wilson's technique) and the rest underwent skeletal examination. Decreases in maternal body weight and food consumption, fetal body weights and fetal viability were observed but were not statistically significant in all studies. The percentage of litters with malformations (mainly skeletal and external) was at least 90% in each study. Although variability in the incidence of individual malformations was observed across studies, the observed malformations were typical of RP, e.g., cleft palate, pinna anomalies and microphthalmia/anophthalmia. Negative control data were provided from corresponding vehicle-dosed groups (either distilled water or 1% methylcellulose/0.2% Tween 80). The current data-base demonstrates RP as reproducible and cost-effective positive control agent in rats and rabbits developmental toxicity studies. Supported by USAMRMC Contract No. DAMD17-92-C-2001.

1004 DAY OF CLOSURE OF THE FOETAL HARD PALATE IN THE S.D. RAT

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The new internationally harmonised reproductive study design, ICH 4.1.3., requires exposure of the female during pregnancy from implantation through to closure of the hard palate. It is therefore necessary to determine the day of closure of the palate to demonstrate that a testing protocol meets the regulatory requirements. Three or four pregnant Sprague Dawley rats (strain Ico OFA.SD (IOPS Caw)) which had been housed and maintained under our standard environmental conditions were necropsied on each of days 15, 16 and 17 *post coitum*. Specific litter parameters were recorded and a total of 54, 48 and 38 foetuses were available for examination in the respective groups. Following external examination each foetus was killed and then examined for closure of the hard palate following fixation; the palate was categorised as open, partially closed or closed. On day 15 of pregnancy, all foetuses had open palates. On day 16, closure was complete in 13/48 foetuses, 29/48 showed partial closure, and the remaining 6 foetuses had open palates.

On day 17, closure was complete in all foetuses. The results indicate that for the strain of Sprague Dawley rat routinely used in reproductive toxicology studies in our laboratory, closure of the hard palate is not complete in all foetuses until day 17 of pregnancy. We recommend, therefore, an exposure period from day 6 to day 17 of pregnancy inclusive for embryotoxicity studies in the rat in order to satisfy the ICH requirements.

1005 A COMPARISON OF THE EARLY EMBRYO EFFECTS OF ETHANOL, 1,1,1-TRICHLOROETHANE, ATRAZINE, 2,4-D, METHYL MERCURY, LEAD AND 2,3,7,8-TCDD

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Traditional methods for evaluating teratogenicity after exposure to a chemical toxicant include evaluating the embryos late in gestation or incubation, or removing the embryos from the dam and exposing cultured embryos to the potential teratogen. In the first situation, a minimum of 2.5–3 weeks are required to run the test, depending on the species. In the second, the embryos do not develop in their natural environment, a factor which can affect the sensitivity of the embryo to the toxicant being assessed. We have been evaluating the early chicken embryo assay for use as a relatively rapid teratogenicity screening method. To evaluate whether the effects of different chemicals can be distinguished using the early embryo assay, we compared the effects of 7 compounds using a dose-response paradigm. The following compounds were tested: lead chloride(0, 0.03, 0.1, 0.3, 1, 5 μg/g egg), methyl mercuric chloride (0, 0.01, 0.1, 0.5, 1, 2.5, 5 μg/g egg), atrazine (0, 1, 3, 10, 30, 100 ng/g egg), 2,4-D methyl ester (0, 0.1, 0.3, 1, 3, 10 μg/g egg), ethanol (0, 0.1, 0.5, 1, 2.5, 8, 10, 14.25, 25, 50, 95% injected into the airsac), 1,1,1-trichloro-ethane (0, 1, 10, 25, 100% in ether injected into the airsac), 2, 3, 7, 8-TCDD (0, 10, 100, 300, 1000 pg/g egg). All compounds were injected into the airsac at the start of incubation. Volumes varied depending on the chemical, with no more than 2 μL injected per gram of egg. Eggs were incubated for 48 (embryonic day [E]2) and 96 (embryonic day [E]4) hours at 99.5°F dry bulb and 86°F wet bulb. At sacrifice, the eggs were opened, the vasculature of the E4 eggs was photographed, the embryos were pre-fixed with about 1 mL of neutral buffered formalin (NBF), removed from the surrounding membranes, and post-fixed in NBF at 4°C until analysis. Embryos were analyzed using the developmental staging outlined by Hamburger and Hamilton (1951) and as used in Henshel et al (1993). The results of these studies indicate that the early embryo assay may be a useful screening method for distinguishing non-teratogens (e.g. atrazine) from teratogens (e.g. methyl-mercuric chloride and ethanol). **This work was supported by a Miles, Inc. Alternatives to Animals Research Grant.**

1006 EFFECTS OF 2,2',4,4',5,5'-HEXACHLOROBIPHENYL AND INDOLE-3-CARBINOL ON 3,3',4,4',5-PENTACHLOROBIPHENYL-INDUCED TERATOGENESIS IN CHICKEN EMBRYOS AND C57BL/6 MICE

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Polychlorinated biphenyls (PCBs) are widespread environmental chemicals. Previous studies have reported that 2,2',4,4',5,5'-hexachlorobiphenyl (hexaCB), a diortho PCB congener antagonized 3,3',4,4',5-pentachlorobiphenyl (pentaCB)-induced teratogenesis (cleft palate) in C57BL/6 mice. In this study, our objectives were two-fold: 1) investigate if hexaCB could antagonize the teratogenic effects of pentaCB utilizing the chick embryotoxicity screening test (CHEST) bioassay, and 2) evaluate the effects of 13C (indole-3-carbinol) on pentaCB-induced teratogenesis in the CHEST assay and C57BL/6 mice. Fertilized hen eggs were injected into the yolk with pentaCB at concentrations ranging from 0.5–12.0 μg/kg egg weight either alone or in combination with hexaCB (10, 25, or 50 mg/kg egg weight) on day 4 of incubation. The experiment was terminated on day 18 and live embryos were subjected to embryonic and histological evaluations. PentaCB alone produced dose-dependent increases in mortality, toxicity and malformations in the embryos. A concentration of 2.0 μg pentaCB/kg resulted in 16% embryonic malformations, 56% edema, and 63% liver lesions. HexaCB, at 25 or 50 mg/kg, antagonized the embryotoxic and teratogenic effects of pentaCB (2.0 μg/kg) in the chicken embryos. Studies have demonstrated that 13C binds to the Ah receptor. Our results indicated that exposure to 13C alone at 2.0 mg/kg in chicken embryos and 200 mg/kg in mice did not result in any toxic effects. Cotreatment of 2.0 μg penta CB/kg plus 2.0 mg 13C/kg (in the CHEST assay) and 1044 μg pentaCB/kg plus 200 mg 13C/kg (in C57BL6 mice) revealed neither agonist nor antagonist effects on pentaCB-induced teratogenic effects. The CHEST bioassay confirmed results in C57BL/6 mice and may be useful to delineate interactions between complex mixtures of environmental toxins (Supported by NIH P42-ES04917).

1007 INTERACTIVE DYSMORPHOGENIC EFFECTS OF ALL-TRANS-RETINOL AND ETHANOL ON CULTURED WHOLE RAT EMBRYOS DURING ORGANOGENESIS

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Whole rat conceptuses (10.5 gestational days) were explanted into a culture medium containing all-trans-retinol (t-retinol, vitamin A_1), ethanol, or combinations of the two alcohols at various concentrations, and were incubated at 37°C for 24 hours. A primary target site affected by t-retinol was the 2nd branchial arch (BA). With initial culture medium concentrations of 3.5 mM of t-retinol, 28% of embryos exhibited underdeveloped 2nd BAs and the effect was concentration-dependent. Incubations with t-retinol alone also caused failure of neural tube closure, underdevelopment/absence of otic and optic vesicles, and failure of normal axial rotation, but were statistically significant only at 10.5–14.0 mM. Ethanol alone produced statistically significant decreases in viability and increases in incidence of embryonic abnormalities at 50 mM but not at 10 or 20 mM concentrations. The embryotoxicity of ethanol appeared less site specific than that of t-retinol. Nevertheless, ethanolelicited developmental abnormalities included underdevelopment of the 1st and 2nd BAs, abnormally open neural tubes, abnormally small or absent otic/ optic vesicles, and incomplete axial rotation in common with effects elicited by t-retinol. Combinations of t-retinol and ethanol produced lower survival rates and higher incidences of developmental abnormalities when compared to the calculated values expected for simple additive effects, i.e., interactive effects in general were greater than additive. Thus, the dysmorphogenic effects were at least additive and probably synergistic but not *antagonistic*. HPLC analyses showed increases in conceptal tissue levels of both all-trans-retinoic acid (t-RA) and all-trans-retinal (t-retinal) after conceptuses were exposed to t-retinol (10.5 mM) plus various quantities of ethanol for 24 hours. These data suggested that the observed greater-than-additive/synergistic effects were not due to the inhibition by ethanol of the biosynthesis of t-RA. Whether the increased levels of t-RA and t-retinal caused the observed greater-than-additive/synergistic effects remains to be elucidated.

1008 GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD): AN EMBRYOPROTECTIVE ROLE IN ENDOGENOUS OXIDATIVE STRESS AND PHENYTOIN TERATOGENESIS

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G6PD catalyses NADPH regeneration, which is critical for cytoprotection against oxidative stress. G6PD deficiency is the most common enzymopathy, affecting over 400 million people worldwide. Endogenous and xenobiotic-initiated oxidative stress may play a role infertility, in utero fetal death and teratogenesis. To determine the embryoprotective importance of G6PD, this study evaluated whether pregnant heterozygous (+/−) and homozygous (−/−) G6PD-deficient dams were more susceptible than homozygous (+/+) G6PD-normal dams to spontaneous and phenytoin-initiated embryopathies. Pregnant dams were either untreated, or given a teratogenic dose (65 mg/kg ip) of phenytoin on gestational days (GD) 12 and 13 (plug = GD 1). Treated dams were sacrificed on GD 19 and implantations (fetuses and resorptions) were evaluated. Untreated dams were allowed to deliver spontaneously. With untreated −/− G6PD-deficient dams, fetal postpartum lethality was almost 3-fold higher than with +/+ G6PD-normal dams ($p<0.0001$). With phenytoin, compared to +/+ dams, both +/− and −/− dams had respective 6- and 5.9-fold, and 7.3- and 10.4-fold increases in phenytoin-initiated fetal resorptions (in utero death) and postpartum lethality, independent of gender ($p<0.0001$), and fetal body weights (g, mean ± SD) from deficient dams (+/−, −/−: 0.8703 ± 0.2186, 0.8127 ± 0.0526) were lower than from +/+ dams (1.0887 ± 0.1237) ($p<0.0004$). This is the first direct evidence that G6PD may be a crucial embryoprotective enzyme against endogenous developmental and xenobiotic-initiated oxidative stress. (Support: Medical Research Council of Canada)

1009 ALTERATIONS IN STRESS PROTEIN SYNTHESIS AND CARDIAC FUNCTION OF CHICK EMBRYOS EXPOSED TO HEAT SHOCK OR ULTRASOUND

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Studies were performed to compare the effects of direct heating to the effects of ultrasound by examining the expression of stress proteins and cardiac function in 72-hour chick embryos. Embryos were heat shocked in a water bath or exposed to continuous wave ultrasound. Embryos were filmed before and after all exposures for functional analysis. After exposures, embryos were labeled with ^{35}S-methionine and the proteins separated by SDS-PAGE. Heat-treated embryos showed the enhanced *de novo* synthesis of three proteins with molecular weights of 21kD, 74kD, and 94kD that demonstrated a dose dependent response to temperature and exposure duration. Western blots probed with monoclonal antibodies against heat shock proteins (hsp) and glucose regulated proteins (grp) identified these proteins as hsp25, hsp70, and grp94, respectively. Ultrasound treatment produced an increase in embryonic temperature and induced the synthesis of the same three proteins but at temperature/time thresholds lower than required by heat treatment alone. Preliminary cinephoto-analysis demonstrated that the ventricular shortening fraction was decreased by heat at a temperature of 45°C, but was unaffected at lower temperatures. However, shortening fraction was decreased by ultrasound at intensities of 100, 250, and 500 mW/cm^2. These data indicate that ultrasound can produce changes in stress protein synthesis and cardiac function that are not induced by direct heating to comparable temperatures.

1010 STRESS PROTEIN SYNTHESIS INDUCED IN CHICK EMBRYOS BY ARSENITE (As), MERCURY (Hg), AND CADMIUM (Cd)

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Cells respond to adverse environmental stimuli by enhancing the expression of specific genes, the products of which comprise a suite of proteins known as stress proteins. The objective of this study was to characterize alterations in expression of stress proteins in chick embryos exposed to As, Hg, and Cd. Embryos (72-hr incubation) were exposed *in ovo* at 37°C. After removing a 1-cm^2 window, solutions of $NaAsO_2$, $HgCl_2$, and $CdCl_2$ were placed directly on the embryo in 10-µl aliquots and the shell resealed. Embryos were exposed for 2 hr to 3, 10, 30, or 100 nmol metal, and to 10 nmol each metal for 2, 4, 12, or 24 hr. Following exposures, embryos were explanted, pulse-labeled for 1 hr with ^{35}S-methionine, and ^{35}S-labeled proteins were analyzed using SDS-PAGE, autoradiography, and densitometry. A dose-related response was observed after 2 hr exposure: As treatment enhanced the synthesis of 21kD, 70kD, and 90kD proteins, Hg enhanced the synthesis of 70kD and 90kD proteins, and Cd the 70kD protein only. These responses were transient; apparent rates of protein synthesis were maximal 2–4 hr after exposure and returned to control rates by 24 hr. The 70kD protein was identified as a member of the heat shock protein family, hsp70. Actinomycin D experiments demonstrated that expression of these proteins is transcriptionally regulated. Enhanced stress protein synthesis preceded metal-induced developmental abnormalities. These data demonstrate that synthesis of stress proteins in avian embryos is rapidly induced after exposure to As, Hg, and Cd. The observed quantitative and qualitative differences in expression of stress proteins may be related to specific mechanisms of developmental toxicity for these metals.

1011 TOXICITY OF THE AROMATIC RETINOID, TTNPB: BINDING AND TRANSCRIPTIONAL ACTIVATION OF THE RETINOIC ACID RECEPTORS

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The aromatic retinoid, (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthylenyl)-1-propenyl] benzoic acid (TTNPB) is 1000-fold more teratogenic than all *trans*-retinoic acid (tRA) in several species. Factors which partially explain the potency of this retinoid include: binding affinities to retinoid nuclear receptors (RARs) in the nanomolar range, reduced affinities for the cytosolic binding proteins (CRABPs) and slow rate of metabolism. The present work investigates additional factors possibly involved in the toxicity of TTNPB including: longer receptor occupancy and increased transcriptional activity of the ligand receptor complex. One factor which might control the length of time a receptor remains occupied is the ligand off-rate. Ligand off-rates were determined in nucleoso fractions prepared from COS-1 cells transfected with cDNA encoding the appropriate RAR subtype. When assayed at 10°C, [^{3}H]TTNPB was displaced from the RARs at a significantly faster rate than that of [^{3}H]tRA. The difference in displacement was less significant at 4°C. These observations are consistent with the 10-fold lower affinity of TTNPB vs. tRA for RARs. The data also suggest that off-rates of TTNPB from the RARs do not explain the greater potency of TTNPB. The ability of TTNPB and tRA to activate the RARs was determined using a luciferase reporter gene transfected into JEG3 cells with the appropriate RAR subtype. The expression of the reporter was driven by a retinoic acid response element (RARE) from the RARβ gene which was incorporated into the reporter plasmid. Using TTNPB and tRA, we are investigating the dose response and time response relationships for gene activation. A concentration-response curve was determined which demonstrated that TTNPB and tRA both activate mRARα, β and γ with EC_{50}s in the nanomolar range.

1012 TERATOLOGIC EVALUATION OF THE TOBACCO CARCINOGEN 4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANONE (NNK) IN VIVO AND IN EMBRYO CULTURE

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The tobacco-specific, in utero animal carcinogen NNK is bioactivated by cytochromes P450, and potentially by peroxidases, to reactive intermediates which can methylate, pyridyloxobutylate and oxidise DNA. There are no studies of NNK teratogenicity. We evaluated NNK teratogenicity in vivo and in embryo culture to determine if NNK may be bioactivated by embryonic peroxidases to a teratogenic reactive intermediate. In in vivo studies, pregnant CD-1 mice were injected with NNK (100 mg/kg ip) or its saline vehicle on gestational days 10 and 11, 11 and 12 or 12 and 13, and teratogenicity was evaluated on day 19. In embryo culture studies, embryos were explanted from pregnant CD-1 murine dams on gestational day 9.5 (vaginal plug = day 1) and incubated for 24 hr at 37°C in culture medium containing either 10 µM NNK or its DMSO vehicle. In vivo, NNK did not significantly increase cleft palates, fetal resorptions or postpartum lethality, nor decrease fetal birth weights. In embryo culture, NNK initiated relatively negligible embryopathy that was limited to slight reductions in crown-rump length and somite development ($p<0.05$). These results suggest that, unlike carcinogenicity, the embryopathic effects of tobacco may not be mediated by NNK. (Supported by the Medical Research Council of Canada)

1013 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN PREGNANT RATS: DISTRIBUTION TO MATERNAL AND FETAL TISSUES

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Since TCDD interferes with fetal development, disposition of TCDD to the mother and developing fetus was investigated. Time-pregnant Long Evans rats received a single oral dose of 1.0 µg [3H]TCDD/kg on gestation day (GD) 8. On GD9 and GD16, dams were sacrificed (n = 5) and tissues (maternal and fetal) were analyzed for [3H]TCDD-derived radioactivity. On GD 9, the highest % dose of [3H]TCDD was localized in the maternal liver (26.3). On GD16 the highest amount of TCDD, while decreased relative to GD9 (13.3%), was still in the liver. This decrease indicates that TCDD has roughly a 7 day half-life and is eliminated more rapidly from pregnant females than nonpregnant females. On GD9, the entire fetal compartment contained 0.01% dose as compared to 0.06% on GD16. This increase may be explained by an increase in fetal mass from GD9 to GD16 as well as differing elimination rates of TCDD between the mother and fetus. On GD9 the fetal compartment contained concentrations of 3H-TCDD that were comparable to maternal kidney and spleen (≅0.05% dose/g). The concentration in both the maternal and fetal compartments dropped slightly by GD16. Within the fetal compartment on GD16, the urogenital tract had the same concentration of TCDD as the fetal liver and the placenta (≅0.03 ng/g). In conclusion, while only 25 pg reached the fetal compartment, resulting in a concentration of 0.12 ng

TCDD/g, this exposure has been shown to cause adverse effects on the developing reproductive system. (This abstract does not necessarily represent EPA policy. CHH supported by NIEHS ES07126).

1014 IN UTERO AND LACTATIONAL 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) EXPOSURE DECREASES EPIDIDYMAL SPERM NUMBER WITHOUT ALTERING SPERM TRANSIT RATE IN THE MALE RAT EXCURRENT DUCT SYSTEM

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Decreased cauda epididymal sperm number (CESN) is one of the most sensitive effects of *in utero* and lactational TCDD exposure, and can be observed in male offspring of rats administered a single maternal dose of 0.064 μg TCDD/kg on gestation day (GD) 15. The reduction in CESN cannot be fully accounted for by decreased spermatogenesis. To explain the decrease in CESN it was hypothesized that TCDD exposure increases the rate of sperm transit through the excurrent duct system (efferent ducts, epididymis and vas deferens), thereby decreasing the number of sperm in the system at any given time. Pregnant Holtzman rats were administered a single oral dose of 1.0 μg TCDD/kg, or vehicle, on GD 15. Offspring were weaned on postnatal day (PND) 21. On PND 50, testicular sperm were labelled in 5 males per litter, from 18 control and 17 TCDD-exposed litters, by injecting 15 μCi [^{3}H]thymidine into each testis, under general anesthesia. Sperm movement through the excurrent duct system was monitored daily 35-56 days post [^{3}H]thymidine injection by isolating sperm from the testis, initial segment, caput, corpus and cauda epididymis and vas deferens, and determining sperm-associated radioactivity within each region. Radioactivity recovered from each region was expressed as a percent of total radioactivity recovered from all regions, and was plotted as a function of PND. Sperm transit times for each region were determined. *In utero* and lactational TCDD exposure significantly decreased CESN by 29%, however it did not alter sperm movement through the excurrent duct system. Time and rate of release of labelled sperm from the testis was not altered by TCDD, nor was transit time through the initial segment (<1 day), caput (3 days), corpus (2 days) or cauda epididymis (9 days). Therefore, TCDD exposure decreases CESN by a mechanism that does not involve an increase in sperm transit rate. (Supported by NIH ES01332)

1015 TRANSGENIC MICE AS A MODEL TO EXAMINE GENE REGULATION BY THE Ah RECEPTOR DURING DEVELOPMENT

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An essential first step in elucidating the role of Ah receptor (AHR) in development is to determine when and where the AHR is functional during development. To specifically localize AHR function we created transgenic mice carrying a reporter gene whose expression is regulated by the activated ligand•AHR complex, and control transgenic animals containing the reporter gene alone (not regulated by the AHR). To construct the transgene we coupled dioxin responsive enhancers (DRE; through which the AHR acts) to a minimal heat shock promoter, hsp68, and the reporter gene LacZ encoding the enzyme β-galactosidase. To determine whether expression of the transgene was induced by AHR agonists, 50 mg/kg β-naphthoflavone (β-NF) in corn oil was administered via intraperitoneal injection to pregnant females on gestational day 11.5. After 24 hr the mice were sacrificed, the embryos excised, fixed and stained for β-galactosidase expression. Control animals were treated with corn oil alone, or received no treatment whatsoever. Transient heat shock treatment was included as a positive control for each strain. In each case, embryos were fixed and incubated with X-gal for 2.5 hours, washed and photographed. Preliminary results suggest that β-NF induces expression of β-galactosidase in embryos bearing the transgene ***DREhspLacZ***, but not in embryos bearing the *hspLacZ* gene. Thus, these transgenic mice provide an *in vivo* model for examinig the ontogeny of AHR activation. Supported by a grant from the MRC.

1016 DEVELOPMENTAL TOXICITY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN MOUSE PREIMPLANTATION EMBRYOS

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent developmental toxicant. Previously, we reported that mouse preimplantation embryos respond directly to 10 nM TCDD by exhibiting precocious blastocyst formation (Reprod Toxicol 7:255–261, 1993). We now report that upon transfer to foster mothers, these embryos display a 70% post-implantation failure rate compared to a 5% rate with the control embryos. In addition, this effect of TCDD was Ah receptor-dependent because it was prevented by concurrent treatment with 1 μM α-naphthoflavone, an Ah receptor blocker. Next, we assesed whether or not preimplantation development was affected by maternal exposure to TCDD since *in vivo* exposure is more environmentally relevant. TCDD (200 μg/kg, i.p., in corn oil) was administered during the onset of oocyte maturation and embryos were flushed from the oviducts at the 2 to 4-cell stage and cultured until the blastocyst stage. Interestingly, blastocyst formation was significantly delayed with TCDD treatment ($n = 110$) compared to controls ($n = 80$, $p \leq 0.003$ using a 2-tailed Student's t test). Blastocyst cell counts were likewise lower with TCDD treatment ($n = 111$) compared to controls ($n = 89$, $p \leq 0001$; 2-tailed Student's t test). Taken together, these data indicate that: 1) TCDD-exposure *in vitro* during preimplantation development leads to post-implantation failure, 2) TCDD-exposure *in vivo* during oocyte maturation and throughout preimplantation development leads to delayed blastocyst formation and fewer cells per blastocyst. Supported by NIH grants ES05409, ES05707, and ES03575.

1017 EXPRESSION OF THE Ah RECEPTOR (AhR) AND CYTOCHROME P-450 1B1 (CYP1B1) IN THE DEVELOPING THYMUS

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The AhR is a member of a subfamily of basic helix-loop-helix transcription factors that mediate development, and circadian and oxidative stress responses. Here, we report on the patterns of expression of the AhR and CYP1B1 in the developing thymus of C57BL/6 mice. CYP1B1 is an AhR regulated gene identified by subtractive hybridization in differentiating keratinocytes. Thymuses obtained from mice at gestation day (gd) 15 through postnatal day 14 were sectioned and subjected to immunohistochemical analysis, using polyclonal antibodies against the murine Ah receptor (α-AhR Bear-1) and human CYP1B1 (1B1-218A). At gd 15, a time of early prothymocyte-induced differentiation of the thymic primordium, the AhR and CYP1B1 showed a diffuse pattern of staining, with expression in both thymocytes and epithelial (TE) cells. At birth and through postnatal day 14, AhR and CYP1B1 localized to cells of the subcapsular and medullary regions, with CYP1B1 being predominantly found in TE cells. Significant nuclear staining of the AhR was observed, consistent with a position that the AhR may be activated by developmental effectors. The intensity and pattern of staining observed at gd 15 suggest involvement of the AhR and CYP1B1 in the early development of the thymic primordium induced by prothymocyte seeding. The expression pattern in the fully developed thymus is consistent with a role in TE/thymocyte interactions required for T cell development and maintenance of thymus function. (Supported in part by grants from the PHS [R01-ES-07009] and AFPA.)

1018 LACK OF CARCINOGENICITY AFTER ORAL TRIMETHYL-PHOSPHATE TREATMENT IN THE RAT

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Trimethylphosphate (TMPO), a possible contaminant in some phosphate esters, is capable of producing a variety of genotoxic effects, however, usually at relatively high dosages. In a life-time bioassay (NTP) with oral administration a higher incidence of subcutaneous fibromas (male rats) and endometrial adenocarcinomas (female mice) was observed, while no increase in other tumour incidences and in each opposite sex was found.

TMPO was administered to Wistar rats by drinking water, and daily doses of 0, 1, 10 and 100/50 mg/kg body weight were applied for up to 30 months to 50 males and 50 females at each dose level. The study design was in

accordance with OECD guideline no. 453. Due to tolerance reasons the initial dose of 100 mg/kg/d was reduced to 50 mg/kg/d after 54 weeks, and the animals had to be sacrificed after 100 weeks in a moribund condition as evidenced by paralysis of the hind legs, weight loss and spontaneous mortality. The other groups were treated for 30 months.

No treatment related increase in the incidence of clinical signs and mortality was observed up to and including 10 mg/kg/d. Pathological changes were confined to males and females at 100/50 mg/kg/d, emaciation and/or myatrophy was present at interim necropsy after one year and even more prominent at termination. The microscopical examination revealed presence of peripheral neuropathy associated with myopathy. The incidence, time of occurrence, spectrum and nature of the observed tumours provided no indication of any relationship with treatment in the dose range investigated.

Thus TMPO was not found to be carcinogenic up to and including 100/50 mg/kg/d, but to be peripherally neurotoxic at 100/50 mg/kg/d. Under the conditions described, TMPO was tolerated without harmful effects at lifetime doses up to and including 10 mg/kg/d.

1019 PREDICTION OF CARCINOGENICITY IN RODENTS USING RULES BASED ON FUNCTIONAL GROUPS AND COMPUTED ELECTRONIC STRUCTURE

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A model was devised to predict carcinogenicity of organic chemicals in rodents from chemical structure. A data set was randomly split into a training and a test set. A model comprised of rules based on hypothesized mechanisms of action, metabolism and partitioning was developed for the training set. Each of the hydrolysis products of esters, and azo compounds were evaluated as individual chemicals. Predictors included logKow, molecular size, delocalizibility on carbon, atomic partial charge, bond angle strain, atomic acceptor delocalizibility, atomic radical super-delocalizibility, the lowest unoccupied molecular orbital (LUMO) energy of hypothesized intermediate nitrenium ion of primary aromatic amines, difference in charge of ionized and unionized carbon-chlorine bonds, substituent size and pattern on PNAs, the distance between hydroxyls over a rigid structure, and the presence of functionalities such as nitroso, thiocyanate and hydrazine. The model correctly classified 96% of the carcinogens in the training set of 306 chemicals. The results of applying the model to test sets will also be presented.

1020 COMPARATIVE DATA FROM THE CD-1 AND B6C3F1 MOUSE IN CARCINOGENICITY STUDIES

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The conduct of mouse carcinogenicity studies is a controversial subject at this time with the Third International Conference on Harmonisation (ICH3) having discussed the use of two rodent species in November 1995. Traditionally the outbred CD-1 Swiss mouse has been utilised for mouse carcinogenicity studies, however, recently a number of organisations have been utilising the hybrid B6C3F1 mouse. Background data studies in the B6C3F1 mouse have been completed within Huntingdon Research Centre to provide data on the bodyweight, food consumption, macroscopic and microscopic pathology (in particular, spontaneous neoplasms) of these animals over a 2 year period. This poster provides comparative data with recently completed CD-1 studies. The B6C3F1 mouse has a longer life-span with survival of 72% for males and 77% for females at 2 years in comparison to values for CD-1 mice of 60% for males and 59% females at 2 years. For decedent animals factors contributory to death show that B6C3F1 females have a higher incidence of lymphoma, whilst males have a higher incidence of hepatocellular carcinoma and urinary obstruction. In contrast, CD-1 mice of both sexes have a higher incidence of amyloidosis and males have a higher incidence of atrial thrombi. For spontaneous neoplastic lesions B6C3F1 mice have higher incidences of liver tumours in both sexes, adrenal cortical adenomas in males and lymphomas in females compared with CD-1 mice.

1021 SUBCHRONIC TOXICITY EVALUATION OF 1, 3-DINITROBENZENE (DNB) IN FISCHER 344 RATS

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Subchronic toxic effects of DNB in male and female rats were evaluated by feeding certified powered Purina diet supplemented with 0, 1, 6, or 30 mg DNB/kg diet, for ninety days. The calculated daily average DNB intake for females and males respectively was 0.07, 0.39, 1.93 and 0.06, 0.35, and 1.73 mg/day/kg BW. Food and water consumption were not significantly different. In the high dose group a significant increase in the relative spleen weight (both sexes), and a decrease in testes weight was noted. Methemoglobin levels were increased significantly in both sexes receiving 6 and 30 mg DNB/kg diet groups. In the same dose groups, the red blood cell count, hemoglobin and hematocrit values were decreased. None of the measured clinical chemistry values were affected. Histopathological examination revealed erythroid hyperplasia in spleen, cytoplasmic droplets in the kidney and seminiferous tubular degeneration in the testes in groups receiving 30 mg DNB/kg diet. From our data the low observed adverse effect level (LOAEL) was 0.35 mg DNB/day/kg BW, based on decreased hemoglobin and increased methemoglobin. Likewise, a no observed adverse effect level (NOAEL) of 0.060 mg DNB/day/kg BW was established. (This abstract does not necessarily reflect US EPA/US Army policy)

1022 DOSIMETRY OF BUTADIENE DIEPOXIDE IN BLOOD AND LUNGS OF RODENTS FOLLOWING VARIOUS ROUTES OF EXPOSURE

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1,3-Butadiene (BD) is a potent carcinogen in B6C3F1 mice but only weakly carcinogenic in Sprague-Dawley rats. To test the hypothesis that mice are more sensitive to BD because they produce more of the highly mutagenic metabolite butadiene diepoxide (BDO_2), rats and mice will be administered BDO_2 and observed for tumors; of special interest is the lung, the most sensitive target organ in mice. Preliminary studies have been completed to determine the dosimetry of BDO_2 in the lung and blood of mice and rats administered BDO_2 by intratracheal instillation, intraperitoneal injection or inhalation. As expected, intratracheal instillation of 26 mg BDO_2/kg delivered a greater dose to the lung than did intraperitoneal injection of the same amount. BDO_2 was cleared rapidly in both species with a half-life of > 5 min. Inhalation of 12 ppm BDO_2 for 6 h achieved lung and blood concentrations in mice that were similar to those observed in both species 30 min after intratracheal instillation of 26 mg/kg. Concentrations in rats were about half those in mice, consistent with the higher min vol/kg body wt in the mouse. A study was performed to determine if a 6-h exposure to 18 ppm BDO_2 would produce acute toxicity in rodents. No toxicity was observed, except focal necrosis of the olfactory epithelium. The amount of BDO_2 received by a mouse lung during a 20-day exposure to 18–20 ppm BDO_2 would equal the total amount received by mouse lungs in 2-yr exposures to 62.5 ppm BD, a concentration known to cause lung tumors. Based on preliminary studies, the carcinogenicity study will be designed with exposures to 0, 10 or 20 ppm BDO_2, 6 h/day, 5 days/wk for 4 wk in animals held for at least 1 year for observation of tumors. [Research supported by the Health Effects Institute and the Chemical Manufacturers Association through FIAs with the US DOE under Contract No. DE-AC04-76EV-01013.]

1023 GENDER-RELATED DIFFERENCES IN CARCINOGENIC EFFECTS OF BENZO(A)PYRENE IN MOUSE LUNG

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Benzo(a)pyrene [B(a)P] is known to produce cancer of lung and other organs in rodents. B(a)P-induced tumor formation has been known to be inhibited upon administration of antioxidants. Since gender-related differences in phase I and phase II detoxification enzymes are known, present studies were designed to investigate the effect of B(a)P and protection by butylated hydroxyanisole (BHA) against B(a)P-induced tumor formation in lungs of male and female CD-1 mice. Male and female mice (7–8 weeks old) were housed separately in four groups and received (a) no treatment (control) (b) BHA alone (c) B(a)P alone and (d) BHA and B(a)P together. B(a)P was administered in a single dose of 3 mg /0.2 ml corn oil by oro-gastric intubation. BHA was mixed as 0.5 g/100g in AIN semipurified diet throughout the experiment for the BHA-treated groups. Menadione (Vitamin K_3) as 1 mg/kg diet was given uniformly, 16 weeks prior to sacrificing the animals. Histological studies showed the highest tumor multiplicity (TM) in B(a)P-treated female lungs amongst all the animals. B(a)P induced 2.57 fold higher TM in females than males. BHA did not induce any tumor formation in males but had a TM of 0.33 in females. BHA did not provide any protection in males but provided 6.75 fold protection in females from B(a)P-induced tumor formation [B(a)P vs. B(a)P and BHA]. There were marked differences

in constitutive and inductive glutathione S-transferase (GST) activities in male and female lungs. BHA caused 4.4 fold induction in males as compared to 1.25 fold induction in females [C vs. BHA]. B(a)P did not cause any marked GST induction in males and females [C vs. B(a)P]. Administration of BHA with B(a)P did not induce GSTs in males but caused 1.5 fold induction in females [C vs. BHA and B(a)P]. Glutathione (GSH) levels were induced 2–3 folds by BHA in both males and females. B(a)P induced GSH by 1.5 fold in males but not in females. Results indicate gender-related differential effects of B(a)P-induced lung tumor formation and the protective effect of BHA in mice and suggest that other factors, besides gender-related differences in GSTs, may also contribute to the differential carcinogenic effects of B(a)P in mice lungs (CA27967 & ESO4147).

1024 THE BIENNIAL REPORT ON CARCINOGENS (BRC): REVIEW AND REVISION OF THE CRITERIA FOR LISTING SUBSTANCES IN THE BRC

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The Biennial Report on Carcinogens (BRC) is prepared in response to section 301 (b) (4) of the Public Health Service Act which stipulates that the Secretary of the Department of Health and Human Services shall publish a report which contains a list of all substances (i) which either are known to be human carcinogens or may reasonably be anticipated to be human carcinogens; and (ii) to which a significant number of persons residing in the United States are exposed. This responsibility has been delegated by the Secretary to Director, National Toxicology Program (NTP). The Director, NTP, initiated a review of the BRC in 1995 to broaden input into its preparation, broaden the scope of scientific review associated with the Report, and provide review of the criteria used for inclusion of substances in the BRC. The criteria was reviewed in open, public meetings by an "ad hoc Working Group" of the NTP Board of Scientific Counselors, and also by the full NTP Board. The NTP Board recommended: 1) the current criteria should be revised; 2) mechanistic information should be used as part of the selection criteria; 3) there should be only two categories; and 4) there should be a formal mechanism for the delisting of substances in the BRC. The criteria was also reviewed by the Public Health Services' Environmental Health Policy Committee (EHPC), and the NTP Executive Committee. The review of the criteria was completed with the submission of a report by the Director, NTP to the Secretary, DHHS which described the review process and contained recommended revised selection criteria and formal listing and delisting procedures for agents, substances, or mixtures for the Biennial Report on Carcinogens. This paper will discuss the criteria review procedure, the revised criteria for selection of agents, substances, or mixtures for listing in the Reports, and the formal listing and delisting procedures for the BRC.

1025 THE DURATION OF THE RODENT CARCINOGENICITY BIOASSAY NECESSARY TO DETECT AGENTS CARCINOGENIC TO HUMANS

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The rodent carcinogenicity bioassay, as conducted in the past, emphasizes sensitivity at the expense of specificity. While the assay detects almost all agents that are carcinogenic to humans, it also responds to many agents that pose little or no carcinogenic hazards to humans. This creates a serious dilemma for both industry and regulators. We have already shown that the bioassay could be made more specific by limiting the daily dose levels of test substance, since all agents known thus far to be carcinogenic to humans display potent activity in rodents. We now suggest that the specificity of the rodent bioassay could be further improved by shortening its duration. Examination of rodent bioassay data for those chemicals known to be carcinogenic to humans reveals that for 20 compounds their tumorigenicity was first detected within 12 months of dosing, and for the 2 remaining compounds within 15 months of dosing. Termination of rodent bioassays at, say, 18 months rather than the normal 24 months, would not only decrease the proportion of positive findings of dubious relevance to humans, but would also facilitate the detection of an increased incidence (earlier onset) of those tumor types that occur in rodents with a high spontaneous incidence. In addition, a study of 18 instead of 24 months would have a higher proportion of survivors and would reduce the complications that arise in interpreting the impact of geriatric diseases on the tumorigenesis process in rodents.

1026 A LIFE TIME FEEDING STUDY OF TAMOXIFEN IN THREE STRAINS OF MICE

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Tamoxifen is an hepatocarcinogen in the rat, but not the mouse. A lifetime feeding study (2 year) with tamoxifen has been achieved for the first time in the mouse strain B6C3F1. This strain is less prone to tamoxifen induced kyphosis than other inbred strains, which limits the length of time that tamoxifen can be administered. Two other strains C57B1/6 and DBA/2 have been fed tamoxifen in the diet for 12 months. In all 3 strains at 3 months, tamoxifen was present in the liver at levels approximately 50 times lower than that seen in rats after similar treatment. Compared to the rat, tamoxifen produced much lower levels of liver DNA damage as assessed by ^{32}P-postlabelling ($\approx$100 adducts/10^8 normal nucleotides in mice, compared with $\approx$3000 adducts/10^8 nucleotides in rat liver, at 6 months). There was no sustained liver cell proliferation and there was no significant increase in the incidence of liver tumours after 2 years tamoxifen administration. These results indicate that, in contrast to the rat, tamoxifen is non-carcinogenic in the mouse because it is present at lower levels in the liver, does not act as an initiator by causing DNA adducts or as a promoter by causing cell proliferation.

1027 RELATIONSHIP OF PHOTOMUTAGENICITY, PHOTOTOXICITY, AND PHOTOCARCINOGENICITY

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Photocarcinogenic compounds alter skin cancer rates by acting as initiators or by affecting promotion or progression. In vitro photomutagenicity studies determine which photocarcinogens have the potential to act as initiating agents. Compounds which have been tested in photomutagenicity studies include psoralens, fluoroquinolones, retinoids, phenothiazines, fuoroquinolines, polycyclic aromatics, acridine dyes, and sunscreen components. Both linear psoralen molecules (8MOP, 5MOP, 3CP, and TMP) and angular psoralens (5-methylangelicin and 4,5'-dimethyl-angelicin) are photomutagenic. These psoralens induce mutations in yeast, bacteria, and mammalian cells, and also induce sister chromatid exchanges and chromosomal damage in mammalian cells or animals. Psoralens which crosslink DNA (8MOP and 5MOP) and those which only cause monoadducts (3CP and 5-methylangelicin) are photomutagenic. Almost all psoralens, when tested, are photocarcinogenic in animals. Several fluoroquinolones, lomefloxacin, ciprofloxacin, and fleroxacin, in conjunction with simulated solar light, induce mutations and chromomosomal damage in vitro. All of these compounds were photocarcinogenic with UVA. Retinoids similar to transretinoic acid enhance photocarcinogenicity in conjunction with simulated solar light. In contrast to the psoralens and fluoroquinolones, retinoids are not photomutagenic. For photomutagens which are also photocarcinogens, a key question in risk assessment is the relative contribution of genotoxicity compared to promoting events, such as hyperplasia due to phototoxicity. The genetic portion can be assessed by comparing drug concentrations which are mutagenic in vitro with plasma/skin concentrations in animal photocarcinogenicity studies, and ultimately with plasma concentrations in humans exposed to the drugs.

1028 ALLYL CATECHOLS WHICH FORM LONG-LIVED QUINOIDS ARE CYTOTOXIC TOWARDS HUMAN MELANOMA CELLS

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The cytotoxicity of quinoids formed from the bioactivation of 3-and 4-substituted catechols has gained recent attention in the treatment of malignant melanoma. The selective formation of these quinoids in melanoma cells could contribute to the cytotoxic properties of the parent catechols through alkylation of cellular proteins and/or DNA, or through the formation of reactive oxygen species especially through redox cycling of *o*-quinones. The high levels of tyrosinase found in melanoma cells prompted us to examine the relationship between tyrosinase-mediated oxidation of a series of catechol derivatives and their cytotoxicity. An increase in the rate of tyrosinase-catalyzed formation of the *o*-quinone did not correlate with an increase in toxicity. Instead it was determined that the compounds which were most toxic to the melanoma cells were those with either long-lived *o*-quinones ($t_{1/2}$ > 89 min) or those demonstrating very rapid rates of isomerization to quinone methides ($t_{1/2}$ < 3 min). Since these rapidly formed quinone methides

are also relatively long-lived, this implies that short-lived highly reactive quinoids are not likely to be responsible for the observed toxicity towards melanoma cells. In summary, the data suggests that cytotoxicity of catechols towards melanocytes depends on the stability of the quinoids which should allow for the development of a chemotherapeutic strategy which is specific for melanoma.

1029 ABERRANT CRYPTS INDUCED BY SHORT AND LONG TERM 3,2'-DIMETHYL-4-AMINOBIPHENYL EXPOSURES IN HOMOZYGOUS RAPID AND SLOW ACETYLATOR SYRIAN HAMSTERS CONGENIC AT THE NAT2 LOCUS

Y Feng, A J Fretland, R J Wagner, W J Martin, T D Rustan, M A Cooley, K J Lee, W K Becker, and D W Hein. *University of North Dakota School of Medicine, Grad Forks, ND.* Sponsor: J E Hulla

Aberrant crypts are considered preneoplastic lesions in colon carcinogenesis. Age-matched rapid and slow acetylator Bio. 82.73/H-*Pat* Syrian hamsters, congenic at the *NAT2* locus, were administered the arylamine colon carcinogen 3,2'-dimethyl-4-aminobiphenyl (DMABP, 100 mg/kg) at first and second week by s.c. injection and sacrificed at 14 weeks (short term) or by s.c. injection once a week for 26 weeks, followed by once every two weeks for up 52 weeks (long term). Whole-mount cecums, colons, and rectums were stained with 0.2% methylene blue, fixed in 4% paraformaldehyde and examined microscopically. Frequencies of aberrant crypt foci were higher in cecums after both short term and long term DMABP exposures. The number of aberrant crypt foci increased significantly from short term to long term exposures in both colon and cecum. Frequencies of aberrant crypt foci in colons of rapid acetylator hamsters were significantly higher than slow acetylators after short term exposure. These results further support aberrant crypts as preneoplastic lesions in colon carcinogenesis and suggest that rapid acetylator genotype is a significant risk factor in colon carcinogenesis from arylamine chemicals. Partially supported by USPHS Grant CA-34627.

1030 METALLOTHIONEIN EXPRESSION IN HUMAN KIDNEY AND IN RENAL CELL CARCINOMA

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Metallothionein (MT) expression has been documented in several human epithelial tumours. This study was undertaken to determine the expression of MT in normal human kidney tissue and in renal cell carcinoma by immunohistochemical staining. Sections from 30 nephrectomy specimens with renal cell carcinoma were stained by ABC technique using a primary polyclonal rabbit antibody against rat liver MT. The expression of MT (intensity & extension) in normal tissue and in the carcinomatous regions were evaluated. The pattern of staining in the tumours was correlated with the tumour grade. The staining intensity was graded on a scale of (0 to 2+) and the extent of staining on a (0 to 4+) scale, representing 25 percentiles. The tumour was graded (I-IV) using Fuhrman grading protocol. In normal tissue, all tubules had staining for MT but intensity and extent were variable. The cells in the interstitium and the glomeruli were negative. Of the 30 tumours, there were 1, 17, 7, and 5 tumours of Fuhrman grade I, II, III, and IV respectively. The mean intensity were (2.0, 1.8, 0.9 and 1.0) for grades I, II, III, and IV respectively. There was a significant correlation between the tumour grade and MT expression. The one way analysis of variance (ANOVA) had a p value <0.05. There was no apparentb correlation between the extent of MT staining and the tumour grade. The positive correlation of MT expression with tumour grade suggests a possible role of MT in renal cancer growth regulation and dedifferentiation. Further studies on the role of MT in the oncogenesis of renal cell carcinoma were warranted.

1031 ONCOGENICITY STUDY OF DI (ISONONYL) PHTHALATE IN RATS

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Male and female F344 rats received 0, 0.05, 0.15, 0.6 or 1.2% Di (isononyl) phthalate (DINP) in their diet for up to 104 weeks. Extra animals received the 1.2% diet for 78 weeks followed by 26 weeks of control diet (recovery group). Cell proliferation and peroxisome proliferation (PP) were determined after weeks 1, 2, 13, 79 and 104. The NOEL for systemic and carcinogenic effects was 0.15% for both sexes. Survival in the high-dose males was reduced. Body weight in the high-dose animals of both sexes was reduced. Absolute and relative increases in liver weights were seen in both sexes of 0.6 and 1.2% groups but there was no pathology in the livers of the 0.6% animals. Hepatocellular carcinoma occurred in the 1.2% rats after week 79. In the recovery rats, livers were comparable to control animals at termination. Absolute and relative increases in kidney weight were seen in females of the 0.6% group and both sexes of the 1.2% group. Tubule cell carcinoma was seen in high-dose males. Kidney enlargement was reversible in the recovery group but not kidney carcinoma. Mononuclear cell leukemia was seen in males and females of the 0.6, 1.2 and recovery groups.

1032 A COMPARATIVE SURVIVAL AND ONCOGENICITY EVALUATION IN FOUR STRAINS OF RATS

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Survival and tumor incidences for rats in carcinogenicity bioassays have been controversial. Reduced survival in Sprague Dawley rats has been a regulatory dilemma because 50% survival at 104 weeks may no longer be possible. The purpose of this study was to evaluate four strains of rats housed under identical laboratory environmental conditions to establish comparative survival, tumor incidence, frequency of non-neoplastic lesions, relative body weight gains, food consumption, and clinical pathology parameters. The data presented will cover study events through 18 months. Fifty animals/sex from Crl: CD®(SD)BR, Hsd: Sprague Dawley®SD™TM, CDF®(F-344)/Crl/Br, and Glx: Han: WIfBR strains were individually housed and provided food (Certified Rodent Diet #5002 pellets) and water *ad libitum*. Animals were observed twice daily for morbidity and mortality. Body weights and quantitative food consumption were measured weekly for the first 13 weeks and monthly thereafter. Hematology and clinical chemistry were evaluated at Weeks 5, 14, 27, and 53. After 104 weeks, all surviving animals will be sacrificed, and tissues will be examined microscopically. Results of the first 18 months revealed a 30% increase in both body weight and food consumption for the Crl: CD rat compared with the Hsd: Sprague Dawley SD strain. Ocular opacities were observed at a high frequency in the CDF(F-344)/Crl/Br strain, which included most animals of both sexes. Palpable masses were increased in females and were predominantly observed in both Crl: CD and Hsd: Sprague Dawley SD rats. These data confirm that strain selection for carcinogenicity bioassays is extremely important.

1033 SUPPRESSION OF 7,12-DIMETHYLBENZANTHRACENE (DMBA) BUT NOT 2-AMINO-1-METHYL-6-PHENYL-IMIDAZO[4–5,f]PYRIDINE (PHIP)-INITIATED MAMMARY TUMORS FOLLOWING A SINGLE LOW DOSE OF DIOXIN

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is antiestrogenic in cultured human mammary cancer cells and suppressed the development of spontaneous and DMBA-initiated rat mammary tumors. TCDD, however, is a promoter of liver, lung and skin tumors and has not been investigated in the mammary tumor model at low doses. Here, female rats were initiated with DMBA (5 mg/kg, 50 days old) or the food mutagen PhIP (75 mg/kg, 10X daily from 47 days) and were given a single dose of TCDD (1 μg/kg) eight days later. Mammary tumors appeared 5–10 weeks after initiation and have incidences of 93% and 73% for DMBA and PhIP at 46 and 33 weeks, respectively. TCDD prevented the appearance of DMBA-initiated tumors for 20 weeks. Tumors began to appear by 24 weeks, but marked reduction of tumor incidence, compared to the DMBA-only group, was observed up to 46 weeks after initiation. By contrast, TCDD had no effect on PhIP-initiated tumors up to 33 weeks following treatment. These data demonstrate that TCDD-mediated suppression of mammary carcinogenesis is an initiator-specific phenomena, a fact which must be taken into account in evaluation of human epidemiological data, and in plans for chemoprevention with antiestrogens.

1034 SCOPOLAMINE HYDROBROMIDE TRIHYDRATE IS NOT CARCINOGENIC TO F344 RATS AND B6C3F$_1$ MICE

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Scopolamine hydrobromide trihydrate is used in transdermal patches for the treatment of motion sickness. Carcinogenicity studies were conducted by

administering 0, 1, 5, 25 mg of the drug / kg body weight in distilled water by gavage once daily, five days per week for up to two years to groups of 60 male and 60 female F344 rats and B6C3F$_1$ mice. Body weights of male and female rats receiving 25 mg/kg and those of male and female mice receiving 5 or 25 mg/kg were lower than those of the controls. Survival of female rats receiving 1, and 25 mg/kg was reduced relative to control. No increase in the incidence of neoplastic and nonneoplastic lesions that could be attributed to scopolamine administration was found in rats or mice. There was a dose related decrease in the incidence of pituitary gland tumors and mononuclear cell leukemia in male and female rats, and of liver tumors in male and female mice. These decreases in tumor incidences may have been related to lower body weight of dosed animals.

1035 COMPARATIVE 90-DAY TOXICITY STUDIES OF 2- AND 4-METHYLIMIDAZOLE IN FISCHER RATS AND B6C3F1 MICE

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2-Methylimidazole (2MI) and 4-methylimidazole (4MI) are identified in ammoniated hay forage for livestocks and in caramel coloring, soy sauce, Worchestershire sauce, wine, ammoniated molasses, caramel-colored syrups, and cigarette smoke. They are formed as a result of interaction of ammonia with reducing sugars. Toxicity studies of 2MI and 4MI were conducted because of their potential for widespread exposure to humans in food and tobacco, lack of toxicity data, and suspicion of carcinogenicity. Groups of 10 male and 10 female Fischer rats and B6C3F1 mice were administered 2MI or 4MI by dosed feed at 0, 625, 1250, 2500, 5000 or 10000 ppm for 90 days. In the 2MI studies, all animals survived. Dose-related reduction in mean body weight gains occurred in rats and mice. Food consumption reductions in male and female rats were dose-related. Feed consumption was similar among male mouse groups and among female mouse groups. No signs of neurotoxicity were observed in rats and mice. Relative liver and kidney weights were increased in treated male and female rats and mice. Serum T3 and T4 levels were decreased and TSH levels increased significantly in male and female rats at ≥2500 ppm. Dose-related thyroid follicular cell hyperplasia occurred in male and female rats and mice. Hepatic UDP-glucuronosyltransferase activities in male rats at 10000 ppm at day 29 were increased. Uterine atrophy and adrenal cortical necrosis in female rats, splenic hematopoietic cell proliferation in male and female mice, and hemosiderin in kidney tubules in male and female mice were observed. In the 4MI studies, 1 male rat at 10,000, 1 female rat at 1250, and 1 male and 7 female mice at 10000 ppm died. Mean body weight gains were reduced in rats and mice in a dose-related manner. Food consumption reductions were associated with body weight effects in rats but were not evident in mice. Tremors occurred in male and female rats and ataxia in female rats at 10000 ppm. Relative kidney, liver, and lung weights were increased in treated male and female rats and mice. Serum T3, T4 and TSH levels were not consistent and logically altered in male and female rats as in the 2MI studies. Hepatic UDP-glucuronosyltransferase activities were reduced at day 29 in male and female rats at 10000 ppm. No thyroid lesions were observed. Hepatocytic vacuolation, testes degeneration, and prostate/uterus atrophy in rats and liver cytoplasmic vacuolization and renal degeneration and dilation in mice were found. The studies demonstrated that 2MI and 4MI have different target organ toxicity and that induction of thyroid follicular cell hyperplasisa in rats and mice by 2MI may be due to enhanced hepatic metabolism of T4 by UDP-glucuronosyltransferase.

1036 PULMONARY CARCINOGENICITY OF CIGARETTE SIDESTREAM SMOKE IN A/J MICE

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A total of 48 male strain A/J mice were exposed to sidestream smoke (SS) generated from Kentucky 1R4F reference cigarettes (Controls: filtered air). Chamber concentrations were 90 mg/m^3 of total suspended particulate, 246 ppm CO and 17 ug/m^3 of nicotine. Animals were exposed for 20 weeks, 6 hours a day, 5 days a week. After 5 months, half of the animals were killed. We found that at this moment 33% of the SS exposed and 11% of the controls had lung tumors (tumor incidence and multiplicity not significantly increased). The remaining animals were allowed to recover in air and were killed 9 months after the begin of the experiment. In the SS exposed groups, 20 out of 24 animals (84%) had one or several lung tumors and the average number of tumors per lung was 1.38 ± 0.22. Both tumor incidence and multiplicity was statistically higher than in controls, where we found an incidence of 38% (9/24) and multiplicity of 0.42 ± 0.14. Analysis of cell proliferation in the respiratory tract showed an initially increased, but then decreasing labeling pattern in the epithelia of the airways and a consistently increased labeling pattern in the nasal cavity. Under appropriate conditions, SS thus produces lung tumors in mice.

1037 A COMPARISON OF THE TUMOR PROMOTING ACTIVITY OF IRRITATING AND NON-IRRITATING DOSES OF MIDDLE DISTILLATES IN MOUSE SKIN

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Middle distillates (MDs), a class of petroleum-derived hydrocarbons which boil between 175–370°C, are tumor promoters in mouse skin. The promotional activity is produced under conditions which also result in local changes at the site of application including chronic irritation and epidermal hyperplasia. The present study assessed whether tumor promotion was a secondary response to these effects by applying equivalent weekly doses of irritating (undiluted; 2x/week) or non-irritating (28% dilution in mineral oil; 7x/week) MDs. Four materials were tested: lightly refined paraffinic oil (LRPO), steam-cracked gas oil (SCGO), a kerosene distillate (KD), and C10-C14 normal paraffins (NP). When applied undiluted, all materials produced severe skin irritation and significant increases in tumor frequency. When LRPO, KD, or NP were applied in mineral oil diluent, skin irritation was generally ameliorated and few, if any, tumors were produced. SCGO was highly irritating and produced a significant increase in tumor frequency when administered in mineral oil diluent. These data indicate that the promotional activity of MDs is likely related to chronic irritation at the application site and not to dose. Thus, when used appropriately in the absence of prolonged irritation, these materials should not present a tumorigenic hazard to humans.

1038 MODULATION OF CARCINOGENESIS BIOASSAY DOSE SELECTION BY MODERATE DIETARY RESTRICTION (MDR) IN RATS: RELATIONSHIP WITH CYTOCHROME P450 (CYP) LEVELS, ENZYME ACTIVITIES AND TOXICOKINETICS (TK)

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Carcinogenesis dose range-finding studies were conducted to determine the effects of MDR [75% ad libitum (AL), measured amount of food, 24 g/day (males) and 17 g/day (females)] on maximum tolerated dose (MTD) selection in Sprague-Dawley (SD) rats [when compared to AL]. Toxicological evaluations indicated that in MDR animals several investigational compounds were better tolerated and had 2–4 times higher MTD and no-observed-effect dose level than those of AL animals. Additional studies using various levels of diet restriction were conducted to assess if the changes in dose-toxicity response were related to TK and metabolism differences, including CYP proteins and related enzyme activities. CYP1A, 2B, 2E, 3A and 4A protein levels were unaffected by various levels of dietary control at 13 and 26 weeks, though CYP3A content was slightly increased in females of a severely diet restricted group (50% AL). CYP-dependent metabolism of a model substrate was likewise not significantly affected, though 50% AL males had slightly higher enzyme activity. Plasma drug or metabolite TK was unaffected by MDR. The results indicate that the greater tolerability by MDR rats to adverse effects of certain drugs is unrelated to metabolism and toxicokinetic differences. Toxicity and metabolism data suggest that when compared to rats on AL feeding, MDR rats appear to be healthier and may be a better animal model for toxicity testing.

1039 THE EFFECTS OF AD LIBITUM (AL) OVERFEEDING AND MODERATE OR MARKED DIETARY RESTRICTION (DR) ON BODY WEIGHT, SURVIVAL, AND CLINICAL PATHOLOGY OF SPRAGUE-DAWLEY (SD) RATS

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A 2-year study with 3, 6 & 12-month interim necropsies is being conducted

with Charles River SD rats (95 sex/group) to evaluate the effects of Purina Rodent Chow fed AL or at moderate (70–75% of AL) or marked (50% of AL) DR. Hematology, clinical chemistry, and urinalyses have been conducted in Study Weeks 4, 7, 12, and 26. The rats fed by moderate and marked DR had significantly lower body weight gains than the AL group. The only urinalyses changes between AL and moderate and marked DR groups was a decrease in urinary pH. Moderate and marked DR rats had a decreased incidence of corneal opacities. Marked DR rats had decreases in leukocyte count, serum glucose, protein, albumin, calcium (females only), and phosphorus (males only), and increases in serum alkaline phosphatase. No significant differences in hematology or clinical chemistry parameters occurred between the AL and moderate DR groups. Moderate and marked DR resulted in no adverse effects on clinical signs. Because the animals in the moderate and marked DR groups had fewer adverse biological effects associated with decreased 2-year survival, the data indicate that moderate DR may be more appropriate as a model for toxicity and carcinogenicity testing.

1040 INHIBITION OF 7,12-DIMETHYLBENZANTHRACENE-INDUCED RAT MAMMARY TUMOR GROWTH BY 6-METHYL-1,3,8-TRICHLORODIBENZOFURAN

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6-Methyl-1, 3, 8-chlorodibenzofuran (MCDF) exhibits relatively high antiestrogenic activity in the female Sprague-Dawley rat uterus and in MCF-7 human breast cancer cell lines but minimal activity for other aryl hydrocarbon (Ah) receptor-mediated responses. The effects of MCDF as an inhibitor of mammary tumor growth was determined in 50 to 60 day old female Sprague-Dawley rats treated with the carcinogen 7, 12-dimethylbenzanthracene (DMBA). After mammary tumors (40 to 60 mm^3) developed, the rats were treated with 10 or 25 mg/kg of MCDF or corn oil for three successive weeks and sacrificed 7 days after the last treatment. In control animals which received corn oil alone, the tumor volume increased from 38 to 399 mm^3 over the treatment period. In animals treated with MCDF (10 mg/kg in corn oil), the average tumor volume increased from 49 to 95 mm^3 and similar results were observed in the 25 mg/kg treatment group. There was considerable variability within the treatment groups; however, in some of the animals treated with MCDF, the tumors could not be detected at the end of the treatment period. In rats administered 10 mg/kg MCDF, hepatic microsomal ethoxy-resorufin *O*-deethylase activity was not induced and only minimal induction (< 2-fold) was observed at the higher dose (25 mg/kg). Ongoing studies are investigating the anti-tumorigenic activities of other alkyl polychlorinated dibenzofurans using rat and mouse mammary tumor models.

1041 ESTIMATES OF THE NUMBER OF LIVER CARCINOGENS IN BIOASSAYS CONDUCTED BY THE NATIONAL TOXICOLOGY PROGRAM

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Estimates are made of the numbers of liver carcinogens in 391 long-term bioassays conducted in rats and mice by the National Toxicology Program (NTP). These estimates are obtained from examination of the global pattern of p-values obtained from statistical tests applied to individual bioassays. Despite the fact that these estimates are biased low, estimates of the number of chemicals of chemicals that were liver carcinogens in specific sex-species groups, as well estimates of the number of chemicals that were liver carcinogens in any sex-species were consistently higher than the corresponding numbers of carcinogens identified by the NTP. Similarly, lower 95% confidence bounds on the numbers of carcinogens in these categories were in some cases higher than the NTP counts. Consequently, this study provides persuasive statistical evidence of more rodent liver carcinogens than were identified by the NTP.

1042 INFLUENCE OF 4-METHYLTHIOBENZAMIDE (MTB) ON RAT LUNG CARCINOGENESIS BY DHPN IN F344 RATS

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MTB was reported to induce high DNA synthesis after severe lung toxicity in rats. Therefore, enhancement of lung carcinogenesis induced by the combined administration of MTB and dihydroxy-di-n-propylnitrosamine (DHPN), a lung carcinogen, was evaluated. F344 male rats were divided into 6 groups, and were given 0.1% DHPN in the drinking water for 2 weeks. Animals were injected with 1 or 0.5 mmol/kg bw MTB ip, 24 hours prior to, 1 week after or 2 weeks after DHPN start. Groups treated either with DHPN or MTB served as controls. Animals were sacrificed at weeks 8, 12 and 16. The results showed that the combined treatment of DHPN with MTB significantly enhanced lung hyperplasia, especially in the group treated with MTB at 24 hours prior to DHPN treatment. Therefore, this protocol may be used as a bioassay to detect the modifying effects of chemopreventive agents on lung carcinogenesis.

1043 FURTHER INVESTIGATIONS OF THE ONCOGENICITY OF DI(2-ETHYLHEXYL)PHTHALATE IN RATS

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F-344 rats were treated with 0, 0.01, 0.05, 0.25, or 1.25% di(2-ethylhexyl)phthalate (DEHP) in the diet for up to 104 weeks. Extra animals received the 1.25% diet for 78 weeks followed by 26 weeks of control diet (recovery group). No progressive treatment-related changes were seen in serum chemistry and hematology parameters after 26, 52, 78, and 104 weeks. Animals were sacrificed after 78 and 104 weeks for histopathology. Peroxisome proliferation (PP) was determined after 1, 2, 13, and 104 weeks. Mean relative liver and kidney weights were higher for the 0.25 and 1.25% groups compared with the controls, while relative testes weights were lower only for the 1.25% group. Organ weight and body weight changes were reversed during recovery. A higher incidence of hepato-cellular carcinoma was observed only for the 1.25% group at 78 weeks and 104 weeks (recovery and continuous treatment groups). The tumor incidence was dramatically reduced in the recovery group. Sustained PP was observed for the 1.25% group throughout the study. PP was also seen in the 0.25% group, but no PP was evident in the recovery group. A clear NOEL was established. In addition, the data indicate that the carcinogenesis process may be arrested by cessation of DEHP treatment late in the animal's life span.

1044 WINGSTAY® 100: ASSESSMENT OF CHRONIC TOXICITY AND CARCINOGENICITY IN RATS

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WINGSTAY® 100 [N,N'diaryl-p-phenylene diamine (CAS # 68953–84–4)] is used as an antioxidant/antiozonant in high durability rubber products. This product (W100) was given in the diet at 3.3, 20 or 120 mg/kg/ bw/day for 52 weeks to male and female F344 rats. Subsequently, rats were allowed to recover post-exposure for 12 weeks. In a parallel study, the high dose of W-100 was tested in an accelerated bioassay (only male F344 rats) set to test initiation and promotion. Diethylnitrosamine (DEN) was used as liver tumor initiator and phenobarbital (PB) as liver tumor promotor. Butylhydroxybutylnitrosamine (BBN) was used as urinary bladder (UB) initiator, and nitrilotriacetic acid (NTA) was used as UB promotor. There was a body weight gain and food consumption reduction in both genders of high dose rats (120 mg/kg bw/d). The mean corpuscular volume (MCV) of the erthyrocytes was increased, and the mean corpuscular hemoglobin concentration (NCHC) was decreased in high dose groups. The total bilirubin in blood and urine was increased while urine pH was decreased. The PCNA labeling index (LI) for liver and urinary bladder was monitored. No change in the hepatocellular LI was evident. In urothelial cells, there was a dose related increase in LI in W100 groups. Compared to values of the positive controls, the LI values in the UB indicated no effect in W100-treated animals. Thus, W100 does not possess direct initiating or promoting properties in either tissues. In the UB, conditions such as low pH may be responsible for the observed urothelial proliferation through a mechanism of local irritation and cytotoxicity.

1045 NEW DIET (NTP-2000) FOR RATS IN THE NATIONAL TOXICOLOGY PROGRAM TOXICITY AND CARCINOGENICITY STUDIES

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The NIH-07 diet with −23% protein, −5% fat and, −3.5% fiber has been the selected diet for the National Toxicology Program (NTP) studies since 1980. Some nutrients of the NIH-07 diet such as: protein may have increased

the severity of chronic diseases. After evaluating a number of experimental diets, a new diet designated as NTP-2000 with −14.5% protein, −8.5% fat and −9% fiber was formulated and evaluated. Groups of 25 male and female F344 rats of 6-weeks of age were fed the NIH-07 or the NTP-2000 diet for 13 weeks and compared for growth patterns, feed and water consumptions, hematology, clinical chemistry and pathologic changes. The growth patterns were essentially same for both diets but the BUN values of rats fed NTP-2000 diet were significantly lower due to lower protein content of the NTP-2000 diet. The 5′ nucleotidase and alkaline phosphatase levels in rats fed NTP-2000 diet were slightly higher possibly due to higher fat content of this diet. However, the serum triglyceride levels in rats fed the NTP-2000 diet were lower possibly due to more fiber in the NTP-2000 diet. Liver and kidney weights of rats fed NTP-2000 diet were significantly lower due to lower protein content of this diet. Nephrocalcinosis (mineralization at corticomedullary junction of kidney) in females, early changes of nephropathy such as renal tubule regeneration in males and cardiomyopathy in both sexes were the common lesions of F344 rats in 13-week studies. The NTP-2000 diet prevented nephrocalcinosis, decreased the incidence and severity of nephropathy and decreased the severity of cardiomyopathy. The NTP-2000 diet is adequate for growth and maintenance of rats and it appears to prevent or decrease the incidence and severity of diet and age associated lesions.

1046 THE EFFECTS OF DIET, AD LIBITUM (AL) OVERFEEDING AND MEASURED FEEDING REGIMENS (MFRs) ON VARIOUS BIOLOGICAL PARAMETERS IN CONTROL SPRAGUE DAWLEY (SD) RATS

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Sponsor: G R Lankas

A 2-year study was initiated in SD rats to compare the effects of AL feeding and MFRs on various biological parameters. Four groups of 120 rats/sex/group were given the following daily rations of UAR AO4C Rodent Chow: Group 1 = AL; group 2 and 3 = approximately 75% of AL food intake; group 4 = approximately 50% of AL food intake. Intermediate results (up to 6 months) are given in the present poster, MFR groups being compared to AL group. Decreased body weight (BW) gain was observed in all MFR groups due to decreased absolute and corrected (for food wastage) food consumption (FC). There were no differences in relative (to BW) FC among the groups showing that the tested MFRs do not reduce the intake of calories or other nutrients per unit of body mass. Changes seen in clinical laboratory parameters in all MFR groups were decreased urine volume and increased urine pH. The following additional changes were noted only in group 4: decreased leukocyte parameters and platelets, decreased serum total protein, albumin, triglycerides, Ca^{++}, and K^{+}, and increased serum alk. phosphatase activity and Cl^{-}. At 3- and 6-month interim necropsies, decreased organ weights were seen in all MFR groups; there were no MFR-related gross and microscopic changes. In conclusion, despite the changes observed mainly in group 4, the tested MFRs have no adverse effects on the health status of SD rats over the first 6 months.

1047 LIVER WEIGHT AND HEPATOMEGALY AS FACTORS IN THE INDUCTION OF LIVER TUMORS IN B6C3F1 MICE

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Analysis of 79 recent National Toxicology Program bioassays have demonstrated that a direct correlation exists between average body (BW) at 12 months on test and liver tumor incidence in control populations of B6C3F1 mice. In order to evaluate whether this relationship could be a result of the impact of BW on liver size, bioassays which measured liver size using interim sacrifices at 3, 9 and/or 15 months on test were evaluated. Comparison of tumor incidence and liver size at different times on test indicated liver weights in experiments with an average liver/BW ratio (LWBWR) (in mg liver weight/g. BW) between 39–50 in males showed a direct correlation of liver weight and liver tumor incidence. For LWBWR outside this range, there appears to be additional tumor burden added by hepatomegaly (defined as a LWBWR >50) and a reduction of tumors in livers with low LWBWR (<39). Thus liver growth proportional to BW accounts for most of liver tumor incidence seen in control mice, with a additional component contributed by hepatomegaly. The data also suggest that any agent or treatment which either increases liver size or induces hepatomegaly will induce liver tumors secondarily to the increase in liver size. Applying these analyses to recent bioassays suggest that secondary mechanisms leading to the induction of mouse liver tumors are important to consider for an appropriate evaluation of risk.

1048 THE EARLY EFFECTS OF AD LIBITUM (AL) OVERFEEDING AND MODERATE OR MARKED DIETARY RESTRICTION (DR) ON BIOMARKERS OF LONGEVITY, DEGENERATION, CELL PROLIFERATION AND CARCINOGENESIS IN SPRAGUE-DAWLEY (SD) RATS

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A 2-year study with 3, 6 & 12-month interim necropsies is being conducted with Charles River SD rats (95 sex/group) to evaluate the effects of Purina Rodent Chow fed AL or at moderate (70–75% of AL) or marked (50% of AL) DR. At 3 & 6 months moderate and marked DR resulted in no adverse effects, but AL-fed rats had pathological changes predictive of poor 2-year survival. Moreover, the marked DR rats had the lowest incidence of corneal dysplasia and most normal patterns of estrous cyclicity at 6 months. The AL-fed rats had the greatest body weight gain, body fat content and increased heart, kidney, lever, thyroid, adrenal, pituitary, prostate, ovary and pancreas weights and the highest degenerative/proliferative lesion incidence. The AL overfeeding-related lesions [(i.e. increased glomerular size (nephropathy), and pituitary size (hyperplasia and tumors), increased and persistent BrdU labeling (cell proliferation of target organs), myocardial fibrosis (cardiomyopathy), etc.], are confounding factors in evaluating the effects of test compounds in this model. In contrast, controlled feeding by 50% to 75% of AL best prevented the early development of these degenerative/proliferative biomarkers predictive of poor 2-year survival and early spontaneous tumor development.

1049 COMPARISON OF GROWTH, SURVIVAL, TUMOR INCIDENCES, AND CLINICAL PATHOLOGY PARAMETERS IN TWO RAT STRAINS ON CALORIC-RESTRICTED OR AD LIBITUMFEEDING REGIMES FOR 104 WEEKS

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Ad libitum feeding of rats in carcinogenicity studies has been criticized because of reduced survival and pathologic changes associated with high caloric intake. To investigate whether restriction of calories prolongs survival and changes the clinical and anatomical pathologic findings in a carcinogenicity study, two rat strains [Charles River(CR)CD Sprague-Dawley derived rats and CR Fischer 344 rats] were fed *ad libitum* (320 rats/strain) or provided diets with up to 50% less calories (140 rats/strain) for 2 years. Weekly body weights and clinical observations were made; interim bleedings for hematology and clinical chemistry evaluations were performed after 4, 13, 52 and 104 wks, and an interim sacrifice was conducted at 52 wks. After 104 wks, both rat strains with reduced caloric intake had fewer adverse signs, up to 37% greater survival, lower body weights and fewer neoplasms and non-neoplastic lesions. Hematology parameters were unaffected. Caloric restriction lowered triglyceride levels and incidences of tumors (up to 52% lower tumor burden). A strong suppression of neoplasms of endocrine origin occurred with caloric restriction, and the onset of leukemia in Fischer 344 rats was delayed. These results indicate the need to further investigate the relationship of increased caloric intake and endocrine-mediated or strain specific tumors.

1050 THE EFFECTS OF INDUCED CHANGES IN BODY WEIGHT DISTRIBUTION ON THE RISK OF LIVER TUMORS IN B6C3F1 MICE IN A BIOASSAY

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B6C3F1 mice used as controls in chronic National Toxicology Program (NTP) bioassays demonstrate a significant direct correlation between average body weight (BW) at various times on test and liver tumor incidence in the assay. These analyses have led to suggestions that dietary intake be controlled

to reduce variability between experiments and provide animals with target BW. To evaluate the ability to extrapolate information from control animals to animals with controlled dietary intakes, as well as animals dosed with agents, some recent chronic studies were evaluated for changes in BW with either controlled food intake or agent dosing. Induced losses of average BW were usually accompanied by a reduction in variance in the BW distribution in an experiment, with the more severe average BW reductions resulting in the largest distribution changes. Evaluation of the consequences of these changes in distribution, using the probability of tumor as a function of BW calculated for individual animals (which is non-linear) demonstrate that: 1) The loss of variance usually resulted in reduced risk of tumor than the change in average BW predicted; and 2) The magnitude of the decline in tumor risk that usually accompanies a decrease in BW variance depends on the average control BW. Changes in BW distribution can result in a 50% decrease in expected tumor incidence, which can significantly modify the tumor incidences estimated from average BW's, and can influence the interpretation of agent carcinogenicity,

1051 IDEALIZED WEIGHT CURVES TO CONTROL BACKGROUND TUMOR INCIDENCE IN B6C3F$_1$ MICE

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Incidence of spontaneous liver tumors in B6C3F$_1$ mice is variable, and directly correlates with body weight at age 12 mo. This creates problems for interpretation of chronic bioassays that use this strain. The use of dietary control to manipulate body growth in B6C3F$_1$ mice used in bioassays to fit a standardized weight curve could address this problem. We used data from NCTR and NTP studies to construct idealized weight curves for male and female B6C3F$_1$ mice. The growth curves predict a spontaneous liver tumor incidence of ca. 15% at age 26 mo. The curves were constructed by initially comparing the growth curves of mice that developed liver tumors within 26 mo to those which had not by 36 mo. This showed that body weight maximally influences liver tumor incidence between ages 16 and 60 wks. Age set-points in this range were established and the mice divided into different body weight groups. Each group consisted of animals within a 2.5g weight range. Mean tumor incidence was calculated for each group and the weight group(s) at each age set-point that exhibited a liver tumor incidence of 15% was determined. The resulting idealized weight curves for each sex fall within the normal range of body weight life-tables of published NTP bioassays. Mice used for both control and treatment groups in future bioassays could be manipulated to fit these growth curves by moderate food restriction or dietary supplementation. A 15% tumor incidence is sufficiently high so as not to compromise the sensitivity of the bioassay, but is low enough to insure that (1) the spontaneous tumors will not obscure chemically-induced liver tumors, and (2) sufficient mice will survive to the termination of the bioassay.

1052 EFFECT OF INITIAL BODY WEIGHT AND MODERATE DIETARY RESTRICTION ON SURVIVAL IN SPRAGUE-DAWLEY (SD) RATS

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The effect of initial and one-year body weights on the two-year survival rate was determined in dietary restricted (DR) and ad libitum (AL)-fed SD rats from Charles River France. DR rats received 70 to 75% of the AL food intake of adult SD rats. The effect of pretest and one-year body weight on the two-year survival rate was analyzed by the Cochran-Mantel-Haenszel test. The dietary restriction, as performed in this study, resulted in a statistically significant ($P < 0.05$) decrease in mean body weight gain and in a statistically significant ($P < 0.05$) increase in the two-year survival rate. The two-year survival rate of DR male and female rats was 80% and 73%, respectively and the two-year survival rate of AL-fed male and female rats was 28% and 38%, respectively. In DR rats, the main causes of death were pituitary and mammary gland tumors. In AL-fed rats, the main causes of death were pituitary and mammary gland tumors, and renal disease. Pretest body weight had no effect on the two-year survival rate, for both genders and feeding regimens. One-year body weight had a statistically significant ($P < 0.05$) effect on the two-year survival rate in AL-fed male rats only. It is concluded that moderate dietary restriction has a beneficial effect on survival by decreasing body weight gain and that initial (pretest) body weight is not predictive of the two-year survival for both sexes and feeding regimens.

1053 DIFFERENTIAL DISPLAY CLONING OF GENES-INDUCED BY PEROXISOME PROLIFERATOR WY14, 643

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Peroxisome proliferators (PPs), a group of non-genotoxic; carcinogens, include hypolipidemic drugs, plasticizers and herbicides. The carcinogenicity of PPs may be mediated by peroxisome proliferator-activated receptor via altered gene expression in target cells. In the present study, primary hepatocytes were obtained from Male Sprague-Dawley rats 3hr after treatment with the PP Wy14, 643. To detect Wy14, 643 early responsive genes, differential display PCR was employed. The method consisted of reverse transcription of mRNA followed by PCR amplification of the cDNA using two 10-mer primers with arbitrary but defined sequences. Using 157 different primer pairs, eight reproducible differentially displayed bands were produced; seven were induced and one was repressed by Wy14, 643. Interestingly, one clone (02–03) has high homology (95% identical) to the mouse transcription factor ZFP-37, a zinc-finger protein involved in differentiation of several cell types. The induction of this clone has been validated by RNase protection assays. Therefore, this peroxisome proliferator-responsive gene may play an important role in regulating downstream events that are involved in altered cell growth and differentiation associated with PP carcinogenesis. (Supported by Zeneca award 2363670 and NIH DK 49009-01A1)

1054 EFFECT OF PROTEIN KINASE C INHIBITORS ON HEPATIC GAP JUNCTIONAL INTERCELLULAR COMMUNICATION BLOCKAGE BY PEROXISOME PROLIFERATORS

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A diverse class of compounds, including hypolipidemic drugs, herbicides, plasticizers, solvents, and analgesic drugs, have been shown to produce hepatic peroxisome proliferation and hepatocellular carcinoma in rodents. Peroxisome proliferators may function through hepatic tumor promotion. Sustained cell replication is often correlated with the formation of hepatic tumors. Protein kinase C (PKC) is associated with hepatic cell turnover. The activation of PKC by phorbol esters results in the inhibition of gap junctional intercellular communication (GJIC). In the present study, the ability of several peroxisome proliferating compounds, including Wy-14,643 and mono(2-ethylhexyl)phthalate (MEHP), to inhibit GJIC through the activation of PKC was evaluated. Administration of Wy-14,643 and MEHP at concentrations of 100, 250, and 375 μM to F344 male rat primary cultured hepatocytes produced an inhibition of GJIC in a dose responsive manner as indicated by the loss of dye-coupling. Coincubation of 100 nM Bisindolylmaleimide I (GF-109203X), a PKC inhibitor, prevented the Wy-14,643 produced blockage of GJIC. Coincubation of 1 μM Chelerythrine, a second PKC inhibitor, decreased the effect of Wy-14,643 on GJIC. The inhibition of GJIC induced by MEHP was also prevented by both of the PKC inhibitors. The results indicate that the inhibition of GJIC by Wy-14,643 and MEHP is likely to be mediated by Ca^{2+}-dependent PKC isoenzymes.

1055 INHIBITION OF GAP JUNCTIONAL INTERCELLULAR COMMUNICATION BY AROCLOR 1254 IN HEPA1C1C7 AND AHR DEFECTIVE HEPA1C1C7 CELLS

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The toxic equivalency factor (TEF) concept has been developed for risk assessment of complex mixtures of dioxins and PCBs, assuming additive effects of the Ah receptor agonists in the mixtures. There is some debate on the predictive value of the TEF concept for carcinogenicity; both *in vivo* and *in vitro* studies indicate also a tumor promotion potential for several di-ortho PCBs with a negligible AhR binding affinity.

In this study induction of P450IAI and inhibition of gap junctional intercellular communication (GJIC) by aroclor 1254 was examined in a mouse hepatoma cell line (hepa1c1c7) and a mutant cell line with a lack of the Arnt protein (BpR).

Aroclor 1254 caused a 5-fold induction of P450IAI (EC_{50} 2.4 μM) only in hepa1c1c7 cells. However, in both the hepa1c1c7 and the BpR cell line a dose dependent inhibition of GJIC (EC_{50} 1 μM) with a maximum of 20% was found. This suggests the existence of an additional non AhR mediated pathway for Aroclor 1254-dependent GJIC inhibition.

The maximum GJIC inhibition observed by Aroclor 1254 was much lower than expected regarding the results with individual compounds. This may be

caused by non additive interaction between individual compounds within the mixture. A rat experiment has been carried out (data in process) to see if similar effects occur *in vivo*.

1056 EFFECT OF THE PEROXISOME PROLIFERATOR CIPROFIBRATE ON LIPID PEROXIDATION AND DNA SYNTHESIS IN CATALASE TRANSGENIC MICE

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Peroxisome proliferators dramatically increase the expression of several genes in the peroxisomal β-oxidation pathway which may result in the increased production of hydrogen peroxide (H_2O_2). Catalase is the major H_2O_2-detoxifying enzyme in the peroxisomes. It has been proposed that increased H_2O_2 levels may be involved in oxidative DNA damage and tumor promotion induced by peroxisome proliferators. To develop a mouse model to address this question, we have generated transgenic mice which exhibit a three fold increase in liver catalase levels over non-transgenic littermates. We have examined the effect of catalase overexpression and administration of the peroxisome proliferator ciprofibrate on hepatic lipid peroxidation and DNA synthesis in a 2x2 factorial study. Ciprofibrate treatment induced the activity of fatty acyl CoA oxidase (FAO) and cytochrome P450 4A1 in both transgenic and non-transgenic mice, though the induction in transgenic mice was slightly lower. Malondialdehyde concentrations were higher in transgenic mice as compared to non-transgenic littermates. Ciprofibrate treatment lowered the malondialdehyde concentrations in transgenic mice to the same level as non-transgenic mice. DNA synthesis in ciprofibrate treated transgenic mice was significantly lower as compared to ciprofibrate treated non-transgenic mice. This result suggests that catalase is effective in controlling the increase in DNA synthesis caused by ciprofibrate. (Supported by ES-05815 and CA-01688)

1057 PEROXISOME PROLIFERATORS: SEX STEROID RECEPTORS AND METABOLISM IN HEPATIC HYPERPLASIA AND NEOPLASIA

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We previously demonstrated alterations in sex steroid metabolism and estrogen receptor (ER) activity in liver hyperplasia induced by peroxisome proliferators (PP). We now test the hypothesis that progression from hyperplasia to liver cancer results in the down-regulation of ER and elevation of androgen receptor (AR). Male rats were fed the PPs clofibrate and BR931 for 3 days to 10 months. Livers were examined for cell proliferation markers, as well as changes in activity and cellular distribution of ER and AR and in key enzymes in sex hormone homeostasis. At all times, liver weight and proliferation markers in treated rats increased. Early exposure to PPs resulted in increased nuclear ER and AR activity. Rats treated for 9–10 months developed tumors, which displayed a marked decrease in ER activity; in contrast, AR activity increased in tumors and in liver surrounding the tumors. Both short-term and long-term exposure to PPs resulted in a dramatic reduction in sex steroid metabolism. This study supports the thesis that in preneoplastic stages such as hyperplasia, there is an elevation of both ER and AR activities, and progression of hyperplasia to cancer results in suppression of ER but maintenance of AR expression. The biological significance of these changes in relation to dedifferentiation and neoplasia remains to be elucidated.

1058 EFFECTS OF WY-14643 (WY) ON PRIMARY HUMAN HEPATOCYTES *IN VITRO*

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WY, a peroxisome proliferator, is a liver carcinogen in rats; however, the potential human carcinogenicity of this chemical is currently unknown. Present work is part of an overall effort designed to assess human risk to WY by comparing the expression of relevant genes in primary rat and human hepatocytes *in vitro*. Hepatocytes were maintained on a basement membrane-like matrix in Waymouth's modified medium containing insulin. Total RNA was isolated from cells following a 48 hr incubation with 10μM WY. Using quantitative reverse transcriptase polymerase chain reaction, the levels of specific transcripts that were increased in rat hepatocytes exposed to WY were determined. WY induced a significant increase in cytochrome P4504A11 (~4–6 fold), and liver fatty acid binding protein (~3–5 fold) mRNA transcripts compared to controls. In contrast, minimal or no change in acyl CoA oxidase and human peroxisome proliferator activated receptors mRNA levels were detected under the current conditions. Finally, preliminary data suggested that WY induced 2–3 fold increase in cell proliferation as determined by immunohistochemical staining for proliferating cell nuclear antigen. These data indicate that primary human hepatocytes are not refractory to WY, and demonstrate the usefulness of human cell cultures in the assessment of human risks to chemicals.

1059 PEROXISOME PROLIFERATORS (PP) ACTIVATE THE PRODUCTION OF MITOGENS BY KUPFFER CELLS *IN VIVO*: STUDIES WITH ANTIBODIES TO TNFα

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We propose that PP activate Kupffer cells (hepatic macrophages) to release mitogens which increase hepatocyte proliferation. The effect of two potent PP, nafenopin (NAF) and WY-14,643 (WY), was evaluated on Kupffer cell activation by monitoring phagocytosis of colloidal carbon in the perfused liver after treatment *in vivo*. Livers from rats treated with either NAF (2 to 24 hr; 100 mg/kg *i.g.*) or WY (24 hr) were perfused with buffer containing colloidal carbon (2 mg/ml). Basal rates of carbon uptake of 136 ± 12 mg/g/hr were increased 5 hr following NAF to 188 ± 12 and remained elevated 24 hr later (203 ± 3). WY also increased rates of carbon uptake 1.8-fold over controls. Serum levels of TNFα, a mitogenic cytokine, and increased tissue levels of TNFα mRNA were detected following treatment, suggesting that TNFα is released in response to WY. To determine whether TNFα is involved in the increase in cell replication caused by WY, rats were injected with polyclonal antibodies to TNFα. One hr later, WY was given, and rats were killed after 5 and 24 hr. Basal levels of cell turnover (BRDU labeling index 1%) were increased with WY about 8-fold above controls at 24 hrs. TNFα antibody inhibited this mitogenic response to values near 2%. Additionally, at 5 hr WY increased microsomal protein kinase C, an enzyme elevated during cell turnover, 3-fold over controls, a response prevented by TNFα antibody. Collectively, this study demonstrates that NAF and WY activate Kupffer cell phagocytosis and that WY acts as an indirect mitogen on hepatocytes *via* TNFα. It is likely that the Kupffer cell, a major source of liver TNFα, is involved in the mechanism of the mitogenic effect of PP (ES-04325).

1060 RAPID PHAGOCYTOSIS OF PARTICLES OF THE PEROXISOME PROLIFERATOR LY 171883 ACTIVATES KUPFFER CELLS

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LY 171883 belongs to a class of compounds known as peroxisome proliferators which includes hypolipidemic drugs, plasticizers, and solvents which characteristically induce peroxisomal and microsomal enzymes involved in fatty acid metabolism as well as cause hepatomegaly and neoplasia when administered to rodents *in vivo*. Although the mechanism by which peroxisome proliferators cause hepatic tumors in rodents is unknown, one hypothesis states that they increase rates of cell proliferation which leads to DNA replication errors and allows for clonal expansion of previously initiated cells. It is well known that a rich source of mitogenic stimuli is activated Kupffer cells, the resident macrophages of the liver. Further, it is known that Kupffer cells are activated when they engulf particles. Therefore, the purpose of this study was to determine if Kupffer cells take up particles of a peroxisome proliferator and become activated. Particles formed from LY 171883 in cell culture medium (DMEM-L) had a mean diameter of about 240 nm. Cultured Kupffer cells rapidly took up LY 171883 particles: about 65 % of the cells engulfed particles in five minutes. Phagocytosis was also dose-dependent; the percent of cells containing LY 171883 increased with the number of particles. Uptake of this peroxisome proliferator significantly stimulated the production of nitric oxide, measured as accumulation of nitrite in culture medium, by about 40% ($p<0.05$). Since Kupffer cells take up particles of LY 171883 and are activated to release nitric oxide, they may also release mitogenic stimuli following phagocytosis and therefore may be involved in the mechanism by which peroxisome proliferators increase cell proliferation and cause liver tumors in rodents. (ES-04325 and 5-T32ES-07126-13).

1061 EXPRESSION OF SPECIFIC MARKER GENES IN RAT HEPATOCYTES EXPOSED TO WY-14,643 (WY) *IN VITRO*

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Although WY is a known rat liver carcinogen, its mechanism of action is not fully understood. Present work was designed to investigate the ability of WY to modulate relevant marker genes in primary rat hepatocytes *in vitro*. Hepatocytes were maintained on a basement membrane-like matrix in Waymouth's modified medium containing insulin. Total RNA was isolated from these cells following treatment with WY. Quantitative reverse transcriptase polymerase chain reaction (QPCR) was then performed to determine the levels of various specific transcripts. Incubation of rat hepatocytes with 10μM WY for 48 hr resulted in a significant increase in cytochrome P4504A1 (~40 fold), peroxisomal acyl CoA oxidase (~30 fold), and liver fatty acid binding protein (~10 fold) transcripts compared to controls. Significant increases in these transcripts were initially detected at 10nM WY and a plateau was reached at 1μM for mRNAs of P4504A1 and acyl CoA oxidase. Finally, an increase in rat peroxisome proliferator activated receptor mRNA (~6 fold) was induced by WY in the presence of dexamethasone (0.1μM) in the medium. Present work indicate that QPCR is a reliable method to simultaneously determine the transcriptional state of several genes in response to chemical agents, and will contribute to understanding the mechanisms involved in WY-induced liver tumorigenesis.

1062 GAP JUNCTION INTERCELLULAR COMMUNICATION (GJIC) STUDIES IN ADULT PRIMARY MOUSE KERATINOCYTE CULTURES TO DETECT POTENTIAL MOUSE SKIN TUMOR PROMOTERS

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There is recent interest to identify short-term (*in vitro*) assays to detect chemicals for tumor promotion potential. One assay involves the inhibition of GJIC, a property shared by many tumor promoters. In the present study, we examined the effects of 7 chemicals on GJIC in adult C3H male mouse keratinocytes: 4 skin tumor promoters of different potencies (12–0-tetradecanoyl-phorbol-13-acetate [TPA], mezerin, benzoyl peroxide [BP] and n-dodecane [DOD]) and 3 non-tumor promoters (phorbol, toluene, and acetone). GJIC analysis (dye transfer/micro injection) was performed at a minimum of 2 dose levels after exposure for 1, 4 and/or 24 hours. All tumor promoters inhibited GJIC: TPA ≥ mezerin > BP > DOD. The non-tumor promoters did not inhibit GJIC at non-cytotoxic doses. The highest no-effect levels for GJIC inhibition were: 0.1 ng/ml (TPA and mezerin), 1.0 μg/ml (BP), 20 μg/ml (DOD), 0.1% (acetone), 100 μg/ml (phorbol) and 1000 μg/ml (toluene). In conclusion, the GJIC assay in mouse keratinocytes may be useful to detect diverse types of mouse skin tumor promoters of different potencies and also discriminate from non-tumor promoters.

1063 GAP JUNCTIONAL INTERCELLULAR COMMUNICATION (GJIC) STUDIES ON 5 PHTHALATE MONOESTERS IN HEPATOCYTES OF FOUR SPECIES: IMPLICATIONS FOR CANCER RISK ASSESSMENT

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The present study examined the GJIC effects in hepatocytes of 4 species (rats, mice, hamsters, humans) for 5 phthalate monoesters which are metabolites of the diesters: di-2-ethyl hexyl phthalate (MEHP), di-n-octyl phthalate (MNOP), two types of diisononyl phthalate (MINP-M, MINP-S), and diheptylundecyl phthalate (M711P). In rat hepatocytes, all 5 monoesters inhibit GJIC. In mouse hepatocytes, 3 monoesters inhibit GJIC (MEHP, M711P, MINP-S) but not MINP-M and MNOP. At non-cytotoxic doses, none of the 5 monoesters inhibited GJIC in hamster or human hepatocytes and in a human liver cell line. This GJIC assay has good cancer predictive potential for phthalates (Toxicologist 15: #1469). In conclusion, these results indicate that, some phthalate monoesters can inhibit GJIC in rat and mouse hepatocytes but not all monoesters show equivalence to MEHP. Moreover, the qualitative species differences (i.e., humans and hamsters insensitive to GJIC inhibition) and other data suggest that phthalate esters pose a negligible (liver) cancer risk for man.

1064 GAP JUNCTIONAL INTERCELLULAR COMMUNICATION (GJIC) STUDIES ON MONO-2-ETHYLHEXYL PHTHALATE (MEHP) IN RAT HEPATOCYTES: EFFECTS OF MODULATORS ON OXIDATIVE STRESS, SIGNAL TRANSDUCTION, PEROXISOMAL BETA OXIDATION (PBOX) and P450 PATHWAYS

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MEHP, a metabolite of diethylhexyl phthalate, inhibits GJIC and increases PBOX in rodent hepatocytes (*Toxicologist 14*:*136). We studied eleven modulating agents for their ability to affect GJIC inhibition of MEHP in rat hepatocytes. A P450 inhibitor, aminobenzotriazole (ABT), protected against GJIC by MEHP. Four agents which are known to protect against oxidative stress also protected against GJIC inhibition: Vitamin E, retinoic acid, pyruvate, and indomethacin. Six agents which are modifiers of the signal transduction pathway also protected against GJIC inhibition by MEHP: cAMP agonist (8 Br-cAMP); PKC/PKA modifiers (H7, H8, calphostin, staurosporine, nicardipine). The PBOX induction by MEHP was studied for 4 agents; it was inhibited by 8 Br-cAMP, but unaffected by H7, retinoic acid, and ABT. In conclusion, the GJIC inhibition of MEHP appears to involve both signal transduction alterations and oxidative stress.

1065 THE ROLE OF GAP JUNCTIONAL COMMUNICATION IN THE TOXICITY OF QUADRICYCLANE, PERFLUORODECANOIC ACID, PERFLUOROOCTANOIC ACID AND JET FUELS JP-8 AND JP-4

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The down-regulation of gap junction intercellular communication (GJIC) by several oncogenes and non-genotoxic chemicals has been implicated in tumor promotion, teratogenesis, and peripheral neuropathies. Therefore, the use of biological assays that monitor GJIC can give invaluable toxicological information of chemicals and be used in predictive risk assessment of exposures to toxicants. The scrape loading/dye transfer technique was used to measure GJIC in WB-F344 rat liver epithelial cells for all chemicals tested. The jet fuels JP-8 and JP-4 inhibited GJIC at a dose of 10 μl/ml medium. Perfluordecanoic (PFDA) and perfluorooctanoic acid (PFOA) inhibited GJIC at concentrations of 100 and 250 μm, respectively. Inhibition was dose-dependent, reversible and occurred at non-cytotoxic levels. Quadricyclane was found to be highly cytotoxic and, did not inhibit GJIC. The incubation time required for inhibition of GJIC by the jet fuels was 20 min, and 5 min for PFDA and PFOA. The short incubation period required for the inhibition of GJIC by perfluoro-fatty acids suggested a membrane-fluidity perturbation mechanism similar to linoleic acid. The perfluoro-fatty acids also inhibited GJIC in a human kidney epithelial cell line, but the jet fuels did not inhibit GJIC in this cell line. This differential effect of jet fuels is a good example of how toxicants, including carcinogens, can be tissue and/or species specific. This research was supported by US Air Force grant #94-NL-196.

1066 THE BIOLOGICAL EFFECTS OF METHYLATED ANTHRACENES ON GAP JUNCTIONAL INTERCELLULAR COMMUNICATION DEPENDS ON THE RING POSITION OF THE METHYL GROUP

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Polycyclic aromatic hydrocarbons (PAHs) are known carcinogens and are derived from the pyrolisis of organic materials, including cigarette smoke. The tumor promoting activity of cigarette smoke has been well documented and methylated anthracenes and phenanthrenes are the predominant PAHs found in cigarette smoke. The down-regulation of gap junction intercellular communication (GJIC) by non-genotoxic chemicals and several oncogenes has been implicated in tumor promotion. Therefore, we determined the effects of the three isomers of methylanthracene on GJIC. Anthracene and 22-methylanthracene did not significantly inhibit GJIC, whereas anthracene methylated in the 1 or 9 position inhibited GJIC with I_{50} values of 22 μM and 36 μM, respectively. Inhibition was dose-dependent, reversible, and occurred at non-cytotoxic levels. Inhibition occurred within 15 min. Western blot analysis showed that connexin43, which is the p2rimary subunit of the hexameric gap junction protein, was hyperphosphorylated in the same fashion

as the well-known tumor promoter, TPA. In conclusion, the biological effect of methylanthracene depends on the ring position of the methyl group, and these inhibitory isomers could play a potential role in tumor promotion of cigarette smoke. This research was supported in part by the NIEHS superfund grant #ESO4911, and the National Food Safety and Toxicology Center at MSU.

1067 DOWNREGULATION OF GAP JUNCTIONAL INTERCELLULAR COMMUNICATION IN MOUSE AND HUMAN LUNG CARCINOMA CELLS AND RESTORATION OF A MORE NORMAL PHENOTYPE BY CONNEXIN43 TRANSFECTION

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Reductions of gap junctional intercellular communication (GJIC) and gap junction protein (connexin) expression are involved in neoplastic transformation and abnormal growth. Several mouse and human lung carcinoma cell lines were examined for GJIC and found to have low levels of dye-coupling (0–30%) and connexin43 (Cx43) expression. Nontransformed mouse lung epithelial cells (E10) and normal human bronchial epithelial (NHBE) cells had high (90–100%) communication and Cx43 expression. The E9 mouse lung carcinoma cell line (13.6% communication) was stably transfected with a Cx43 cDNA expression plasmid and GJIC was enhanced to 81.9% (E9–2 cells) and 41.6% (E9–3 cells) and Cx43 expression was similarly elevated. The *in vitro* population doubling rate of these cells was not changed, but the saturation density was reduced approximately 33% in both transfected lines. The tumorigenicity of the Cx43-transfected E9 cells was also reduced. Whereas the E9 parental cells formed tumors within 2 weeks in syngeneic Balb/c mice with a 100% incidence (6/6 mice), the highly communicating E9–2 clone had a 0% incidence of tumor formation (0/10) and the moderately communicating E9–3 clone had a 40% incidence (4/10). These studies support a role for GJIC in limiting the saturation density of lung carcinoma cell growth *in vitro* and tumorigenicity *in vivo*. They also suggest that Cx43 is a tumor suppressor gene in lung epithelial cells. (Supported by CA57612, CA33497, and the Ohio Cancer Research Association).

1068 VALUE OF LIVER TISSUE DOSIMETRY DATA TO DESIGN AND INTERPRET *IN VITRO* (HEPATOCYTE) GAP JUNCTION STUDIES WITH PEROXISOMAL PROLIFERATING AGENTS

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Peroxisomal proliferating agents (PPA), such as trichloroethylene (TCE) and di-2-ethylhexyl phthalate (DEHP), are non-genotoxic liver carcinogens in mice (DEHP, TCE) and/or rats (DEHP). In vitro studies employing hepatocyte cultures are being used for PPA with a focus on inhibition of gap junctional intercellular communication (GJIC). Inhibition of GJIC has been proposed as a promising assay for tumor promoters. DEHP is readily hydrolyzed to the monoester (MEHP) in vivo and MEHP is the main chemical found in rodent liver. DEHP (1000 uM) failed to inhibit GJIC inhibition in rat/mouse hepatocytes, most likely due to poor hydrolysis to MEHP while MEHP (150–500 uM) inhibited GJIC in rat/mouse hapatocytes and was readily metabolized in this system. TCE is extensively metabolized to trichloroacetic acid (TCA) in mice but not rats (in vivo, hepatocytes). TCE and TCA inhibited GJIC in mouse hepatocytes but not in rat hepatocytes. In conclusion, metabolic and tissue dosimetry data on PPA can assist in design and data interpretation on whether GJIC effects in vitro are due to parent chemical or metabolites and also provide a sound basis for species differences.

1069 EFFECT OF CI-924 ON PEROXISOME PROLIFERATION, P4504A1 INDUCTION, CELL PROLIFERATION AND APOPTOSIS IN RATS AND MICE

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CI-924 has previously been shown to be a peroxisome proliferator in rats and mice, and to increase the incidence of hepatic tumors in male and female B6C3F1 mice, but not in male and female albino Wistar rats. Male and female B6C3F1 mice and albino Wistar rats were treated with CI-924 at doses of 0, 25 and 75 mg/kg for 1, 3, 7 and 28 days in an attempt to identify species differences potentially related to tumorigenicity. Early and sustained hepatic peroxisome proliferation, determined by acyl CoA oxidase, carnitine acyl transferase activities and P4504A1 induction, occurred in mice and rats of both sexes at 25 and 75 mg/kg CI-924. The effect of CI-924 on glutathione, glutathione peroxidase and glutathione S-transferase were small and varied with sex and species. Increased cell proliferation and decreased apoptosis have both been linked to carcinogenicity. However, while cell proliferation was increased by CI-924 in both species, the largest increases were observed in male rats, and apoptosis was decreased in male rats but was unchanged in male mice. In conclusion, species differences in CI-924 induced peroxisome proliferation, microsomal enzyme induction, cell proliferation and apoptosis were not an explanation for different tumorigenicity findings in mice and rats. A.H.H. is the recipient of a Medical Research Council of Canada postdoctoral fellowship.

1070 DIFFERENTIAL GENE EXPRESSION IN MOUSE LIVER INDUCED BY EXPOSURE TO DIETHYLHEXYL PHTHALATE

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Diethylhexyl phthalate (DEHP) is a widely used plasticizer which induces peroxisome proliferation in rodents. Prolonged exposure to DEHP results in a variety of toxic effects, the most significant of which appear to be male reproductive toxicity in rat and liver cancer in rodents. Accompanying these toxic effects is the induction of a number of genes within the liver, particularly those genes involved in peroxisomal beta-oxidation and the cytochrome P450IVA family of genes. In order to further explore what genes may be altered by DEHP exposure, mRNA differential display was performed using total RNA from livers of male C57B6 mice that were treated with either 0 or 2.0% DEHP in their diet. In doing so, a number of partial cDNAs representing messages that are potentially differentially expressed have been isolated. Completion of the RNase protection assay and northern analysis have shown that one of these cDNAs represents a message that is potentially differentially expressed. Sequencing analysis is currently being performed to compare this partial cDNA to previously isolated genes.

1071 NOVEL TARGETS OF PEROXISOME PROLIFERATOR ACTION: α_{2u}-GLOBULIN AND 17β-ESTRADIOL DEHYDROGENASE

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In an effort to better understand the mechanism of peroxisome proliferator chemical (PPC) carcinogenicity and other effects in the rodent, we used the differential display technique to isolate genes that are regulated by exposure to the PPC Wy-14,643 (WY) in F344 rat liver. We identified a total of 19 genes whose transcript levels were modulated by WY exposure. One of the down-regulated genes was shown by sequencing to be α_{2u}-globulin (α2u). Both the mRNA and protein levels of α2u in liver of male F344 rats were down-regulated > 10 -fold at 3 wk of feeding WY at 0.1% in the diet. The full-length cDNA of an up-regulated gene was cloned and sequenced. The full length clone revealed 81% amino acid similarity to the porcine 17β-estradiol dehydrogenase, type IV (17β-EDH) from a 3' fragment (clone 25). The rat 17β-EDH exhibited homology to short-chain alcohol dehydrogenases and sterol carrier proteins. The hepatic mRNA and protein levels of 17β-EDH increased 5–10 -fold in F344 rats after dietary exposure to WY (0.1%) or gemfibrozil (0.6%) for 3 wk (males; females not done) and gavage administration of WY (50 mg/kg/d) or di(2-ethylhexyl) phthalate (2000 mg/kg/d) for 3 days (males and females). In the rat hepatoma cell line H4IIEC3, in-vitro exposure to WY (250 uM) also caused up-regulation of 17β-EDH. Immunolocalization experiments showed that 17β-EDH is primarily peroxisomal. As the porcine enzyme oxidizes active estradiol to the inactive estrogen, estrone, PPC-induced expression of 17β-EDH may modulate the activity of estrogen receptor in rats.

1072 SPECIFICITY OF THE EFFECTS OF ISOMERIC CLOFIBRIC ACID ANALOGS ON HEPATIC PEROXISOME PROLIFERATION

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Agents that cause peroxisome proliferation include a diverse group of chemical compounds; however, very few reports have examined the role of stereochemistry on this phenomenon. We have studied the effects of a series of asymmetric 2-(4'-chlorophenoxy)acetic acid [CPAA] analogs of clofibric acid on the rat peroxisome proliferator-activated receptorα (rPPARα) transfected into CV-1 and HepG2 cells, using a transactivation assay. In both cell types, the n-propyl and phenyl analogs of CPAA exhibited a high degree of stereoselectivity (S > > R), whereas the methyl and isopropyl isomers showed small differences in potency (S ~ R). Interestingly, the stereoselectivity was reversed for the benzyloxy derivatives of the methyl analog of CPAA (R > S). The same order of stereoselectivity, as observed for these analogs on PPARα) activation, was maintained for the induction of fatty acyl CoA oxidase, an enzyme marker for peroxisomes, in rat hepatoma (H4IIEC3) cells. Thus, these data suggest that an enantioselective interaction of the drug molecule with a specific site on a protein, possibly PPARα, occurs as an initial event leading to an increase in enzyme activity. We conclude that these CPAA analogs may be used as probes to elucidate the mechanism of action for this chemical class of peroxisome proliferators.

1073 THE IDENTIFICATION OF PROTEINS THAT ASSOCIATE WITH THE RAT PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR ALPHA (PPARα)

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The rat peroxisome proliferator-activated receptor α (PPARα) is a member of the steroid hormone receptor (SHR) superfamily. PPARα has been implicated in mediating the biological effects of peroxisome proliferators by altering gene expression of target genes. The activity of SHRs can be modulated in part by interacting with cellular proteins. The interaction of PPAR with cellular proteins has not been extensively examined. To further characterize PPPAR's mechanism of action we screened a cDNA expression library (constructed from a rat hepatoma cell line) with recombinant PPAR (interaction cloning) to look for mammalian proteins that associate with PPAR. Eight clones, PPAR-associated proteins (PAPs), were isolated using this method, subcloned into the pZL1 vector, and sequenced. Initial sequence analysis demonstrated no significant homology with known proteins. Protein-protein interactions were confirmed using co-immunoprecipitation and yeast two-hybrid techniques. The information gathered on PAPs may shed new light on the signal transduction pathways utilized by PPAR. (Supported by Searle Fellowship and NIH DK49009-01A1)

1074 COMPARATIVE TOXICITY OF NIXTAMALIZED AND WATER-EXTRACTED FUSARIUM MONILIFORME CULTURE MATERIAL

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Fumonisins are mycotoxins produced by *Fusarium moniliforme* and *F. proliferatum*. Fumonisins are found on corn and in corn-based feeds and food products. The most prevalent homologue, fumonisin B1 (FB1), is a water soluble molecule that is toxic to a variety of species. There is evidence suggesting that fumonisins may also be an etiological factor for human esophageal cancer. Masa flour for tortillas is made from corn by nixtamalization, a process which hydrolyses fumonisins. The purpose of this study was two-fold: to determine the effects of nixtamalization on the *in vivo* toxicity of corn experimentally molded with *F. moniliforme* (culture material = CM) and to compare the effectiveness of nixtamalization and water extraction for detoxifying the CM. Male rats (n = 10/group) were fed diets containing 5% weight equivalents of (a) CM, (b) nixtamalized CM (NX), (c) water-extracted CM (WE), or (d) sound corn (SC) (control group) for 4 weeks. Water extraction removed most of the FB1 from the CM; the CM and WE diets contained 71 and 8 ppm FB1, respectively. No detectable FB1 remained in the nixtamalized CM, but the NX diet did contain 58 ppm hydrolyzed FB1 (HFB1). Body weight gain of the four groups differed significantly ($p<0.05$) in the order SC>WE>NX>CM. Serum chemical, organ weight, and histopathological findings in CM, NX, and WE-fed groups were consistent with FB1-induced hepato- and nephrotoxicities. Liver lesions were found in all CM and NX-fed rats, although they were significantly less severe in the latter group. In contrast, significant liver effects were not found in the WE-fed group. Nephropathy was found in all CM, NX and WE-fed rats and was of similar severity in each of these groups. These results suggest that the *in vivo* effects of nixtamalized CM containing HFB1 were similar to those of the FB1-containing CM and that nixtamalization was a less effective than water extraction for reducing the toxicity of FB1-contaminated corn.

1075 GLUTATHIONE DEPLETION BY SATURATED ALDEHYDES IN CULTURED RAT LUNG TYPE II CELLS

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Exposure to aldehydes from motor vehicle emissions may rise with the increased use of oxygenated fuels. Emissions of formaldehyde increase with addition to gasoline of methyl-*tert*-butyl ether whereas acetaldehyde emissions increase with additions of ethanol or ethyl-*tert*-butyl ether. The effects the first four members of a homologous series of saturated aldehydes (formaldehyde, acetaldehyde, propionaldehyde, and butyraldehyde) on intracellular glutathione (GSH) levels, cell viability, and cell volume were investigated using an adult rat lung type II cell line. Cell suspensions were treated for 30 min with three concentrations of each of the aldehydes. Cells were exposed to trypan blue, analyzed for cell viability, and measured for volume using an image analysis system coupled with a light microscope. Cell lysates were analyzed for GSH by capillary electrophoresis using micellar electrokinetic chromatography. High (mM) concentrations of the aldehydes were required to deplete GSH. At 100 mM, the order of GSH depletion was butyraldehyde > formaldehyde > acetaldehyde = propionaldehyde, of cell viability decrease was butyraldehyde > propionaldehyde > formaldehyde > acetaldehyde, and of cell volume increase was butyraldehyde > propionaldehyde > acetaldehyde > formaldehyde. Unlike α,β-unsaturated aldehydes, where chemical reactivity with GSH correlates with *in vitro* and *in vivo* toxicity, a simple structure-activity relationship did not result with this homologous series of saturated aldehydes. The mechanisms of toxicity of saturated aldeyhdes may be a combination of loss of cell osmoregulation and chemical reaction with GSH. These results can, however, be useful in estimating chemical toxicity from *in vitro* data.

1076 PRECLINICAL SAFTEY EVALUATION IN RATS OF DESIGNER OILS HIGHLY ENRICHED IN ARACHIDONIC ACID AND DOCOSOHEXAENOIC ACID

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Arachidonic acid (ARA) and docosohexaenoic acid (DHA) are important in human brain and retina development and there is growing evidence showing the importance of these fatty acids in infant nutrition. Triglyceride oils, highly enriched in ARA (ARASCO®) and DHA (DHASCO®) were evaluated in very high dose (20 g/kg) acute and 4-week daily gavage dosing studies in rats. Formulaid®, a combination of ARASCO and DHASCO, was also tested in the 4-week study. ARASCO, DHASCO, and Formulaid were found to have no observable adverse effect levels of more than 2.5, 1.25, and 3.75 g/kg/d, respectively. These represent a 50-fold safety margin over intended use in infant formula. Survival, clinical signs, body weight gain, food consumption, organ weights, clinical chemistry and histopathological evaluations failed to show any differences in animals administered these oils when compared to animals administered equal amounts of high oleic sunflower oil. The bioavailability of ARA and DHA for each of the tested oils was verified by detection of increases in these fatty acids in brain, heart, and liver. Because these oils are enriched in only a single bioactive fatty acid, and they have shown to be safe, they may offer a new source of these fatty acids in specialty foods such as infant formula.

1077 SITE-DIRECTED DERIVATIZATION OF FUMONISIN B_1 CONJUGATES

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Fumonisin mycotoxins are produced by *Fusarium moniliforme*, which grows primarily on yellow corn. The fumonisins are associated with possible human

and rodent carcinogenicity, and cause a variety of human and animal toxicities. Their presence at variable levels in domestic food has generated regulatory interest in the detection, quantification and confirmation of fumonisins in foods. Fumonisin B_1 (FB1) was derivatized with 9-fluorenylmethylchloroformate (Fmoc-Cl) to enable detection of FB1 by hplc/sensitive spectrometric detection methods, to provide a blocking group for the primary amine during coupling reactions, and to facilitate determination of substitution levels of FB1 on immunogen conjugates and solid phase antigens. The Fmoc-FB1 derivative was purified from the crude reaction mixture by extraction with toluene in high yields. Reaction products were identified and verified by electrospray mass-spectrometry. The Fmoc-FB1 derivative was covalently coupled to keyhole-limpet hemocyanin, rabbit serum albumin, and amino-derivatized column packing material through the tricarballylic carboxyl groups using 1-ethyl-3-(3-dimethylaminopropyl)carbodimide-HCL. This results in leaving the NH2 end (domain A) exposed distal to the carrier protein. The Fmoc protecting group was then released from the FB1-NH2 group by the addition of piperdine. Substitution levels of FB1 on protein were determined quantitatively by monitoring the release of Fmoc by LC with fluorescence detection. Conjugates were subsequently used for immunoassay development.

1078 PRODUCTION AND CHARACTERIZATION OF ANTISERA SPECIFIC FOR THE C1-15 DOMAIN OF FUMONISIN B1

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Fumonisin mycotoxins are produced by *Fusarium moniliforme*, which grows primarily on yellow corn and is found in domestic foods. To date, immunochemical assays to detect fumonisin B_1 (FB1) have relied on antibodies prepared against immunogenic conjugates linked through the C2 amino. Here we report the production of high titer polyclonal rabbit antisera prepared against FB1 conjugates in which the amino end of FB1 (domain A) was oriented distal to the carrier protein. Immunogenic conjugates were prepared by coupling 9-fluorenylmethylchloroformate (Fmoc-Cl) blocked FB1 to keyhole limpet hemocyanin (KLH) through the tricarballylic end (domain B) using 1-ethyl-3-(3-dimethylaminopropyl)carbodimide-HCL resulting in the NH2 end (domain A) exposed after removal of the Fmoc protection group. Domain A specific polyclonal antisera was produced by immunizing New Zealand White rabbits with 500 μg of the KLH conjugate in complete Freunds adjuvant and then boosting every 4 weeks with 500 μg of the conjugate in incomplete Freunds adjuvant. Non-competitive ELISA revealed that this antiserum recognized the FB1-RSA conjugate but not RSA alone. Competitive ELISA run at serum dilutions of 1:20,000 using structurally related inhibitors demonstrated a ranked inhibitory potency of FB1<FB2<<aminopentol≈ FB3<<Fmoc-FB1<<<<tricarbyllic acid (no inhibition detected). The 50% inhibitory concentration for FB1 was approximately 2 pmol per well. Collectively this data indicates that the major epitope recognized by this antisera is comprised of antigenic determinants in the Cl-15 portion of the FB1 molecule (which contains the putative biologically active site).

1079 THE TWENTY-EIGHT DAY ORAL TOXICITY OF ENDOGLUCANASE CELLULASE CONCENTRATE UF IN RATS

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Historically, enzymes have been used in food preparation long before their actual "discovery." With the development of modern enzymology, there has been a significant increase in the use of isolated and purified enzymes both in foods and food processing procedures. Cellulose is the most plentiful organic material and is broken down by cellulolytic enzymes. One of this class of enzymes is endoglucanases or 1, 4-B-D-glucan-4-glucanohydrolase (EC 3.2.14), which hydrolyzes the B-1-4-glycosidic bonds of cellulose in a random fashion. As part of the safety assessment of Endoglucanase Cellulase UF Concentrate, an oral toxicity study was performed in Sprague-Dawley rats (10/sex/group). Animals received daily doses of 0, 40, 200 or 1000 mg/kg/day for 28 days. No test article related effects were observed with regard to body weights, weight gains, food consumption values, clinical chemistry, ophthalmology or hematology. Similarly, gross necropsy and histopathological evaluation revealed no adverse effects. Based upon the results of this work, a NOEL for Endoglucanase Cellulase UF Concentrate of 1000 mg/kg/day was recommended.

1080 SPINOSAD INSECT CONTROL AGENT: LACK OF EFFECTS IN A ONE YEAR NEUROTOXICITY SCREENING STUDY IN RATS

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This study was conducted according to USEPA-FIFRA Guideline 83-1 (1991), and was conducted concurrently with a two-year chronic toxicity study. Ten Fischer 344 rats/sex/dose were fed Spinosad at 0, 0.005, 0.02, 0.05 or 0.1% of the diet. Rats were evaluated at pre-exposure and after 3, 6 and 12 months of exposure. Tests included the functional observational battery and measurements of grip performance, landing foot splay, and automated tests of motor activity. After completion of 12 months of exposure, comprehensive neuropathologic examination of perfusion fixed central and peripheral nervous system tissues was conducted on 5 rats/sex/dose.

Spinosad did not affect any of the neurotoxicological parameters in this study.

1081 INABILITY OF OLESTRA TO ADVERSELY EFFECT HUMAN INTESTINAL MICROFLORA

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Olestra, a fat replacer made from sucrose and edible fat, is not absorbed or metabolized and will pass intact through the GI tract. The effect of consuming 0 or 24 g/day olestra on the GI microflora was assessed in 97 humans consuming once daily for 28 days either a moderate (7 g) or high fiber (24 g) meal by measuring indicators of microbial metabolism in expired breath and feces, and microbial fecal counts. Olestra had no significant effect on breath hydrogen or breath methane production and was associated with increases in the concentration of some of the individual fecal short chain fatty acids (SCFAs) such as butyric acid. The individual values for breath hydrogen and fecal SCFAs in subjects eating olestra were within the range of normal variation associated with dietary alterations, indicating that olestra did not have an adverse effect. Olestra had no significant effect on the fecal excretion of betaaspartylglycine or mucin, fecal tryptic activity, or either total microbial viable counts or direct microscopic cell counts in feces. As expected due to partitioning of cholesterol and bilirubin into olestra, fecal concentrations of urobilinogen and coprostanol were reduced. In conclusion, olestra had no effect on intestinal microbial metabolic activity different from those which occur following consumption of common constituents of the diet.

1082 TOXICITY OF FUMONISIN B1 (FB1), ERGOVALINE AND ERGINE IN RATS

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Fusarium moniliforme is a common mold of cereal grains that produces fumonisins and Acremonium coenophilum is an endophyte of fescue grass that produces ergot alkaloids (ergovaline, ergine, etc.). There is a potential in livestock fed grain concentrates and grazed on fescue pastures for simultaneous exposure to fumonisins and fescue toxins. In this study the sensitivity of 5-week old rats (6 rats/group) to FB1 (semipurified and crude), ergovaline, ergine, and their combination was determined during a 2-week oral or intraperitoneal exposure period. The daily doses of FB1, ergovaline and ergine were 15 mg/kg feed, 0.09 mg/rat and 0.04 mg/rat, respectively. The group receiving combination were administered 1/3 of the dose of each toxin. Control rats received vehicle or bromoergokryptine. FB1 alone caused a decrease in serum antibody titer to sheep red blood cells (an indicator of humoral immune response) and there was an increase in liver sphinganine to sphingosine ratios when administered alone and in combination with fescue toxins. There were no changes in feed consumption, rectal temperature, body weight, organ weight, serum total protein, liver enzymes and creatinine concentrations between different groups. A rat administered ergovaline had necrosis of the distal end of the tail. These results indicate that FB1 caused changes in humoral immunity and sphingolipid concentration at doses insufficient to cause liver or kidney damage and that combination with fescue toxins had no effect on FB1 toxicity.

1083 SUBCHRONIC (ORAL) COMBINED NEUROTOXICITY AND TOXICITY STUDY OF ARASCO® AND DHASCO® IN THE RAT

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This study was designed to assess the toxicity of ARASCO® and DHASCO® (single cell microbial oils containing 40% arachidonic acid and 40% docosahexaenoic acid respectively) when administered orally, via gavage, to 160 Sprague-Dawley CD® rats (20/sex/group) at dose levels of 1.0 and 2.5 g/kg/day of ARASCO® and 0.5 and 1.25 g/kg/day of DHASCO® for a period of 90 days. Two groups of control animals received untreated standard laboratory diet or the vehicle at the same dose volume as administered to the treated animals. Physical observations, ophthalmoscopic examinations, body weight, food consumption measurements and neurobehavioral assessments were performed on all animals pretest and at selected intervals during the study. Clinical chemistry and hematology parameters were evaluated at study termination. After 90 days of treatment, all survivors were sacrificed, selected organs were weighed and organ/body weight and organ/brain weight ratios calculated. Complete macroscopic postmortem examinations and histopathological evaluation of selected tissues were conducted on all animals in the vehicle control groups. Mean serum alkaline phosphatase values for males and females receiving both doses of DHASCO® were elevated in a non-dose dependent manner at study termination. No other changes in serum enzymes were seen and no morphologic changes were seen microscopically. These differences were not considered a toxic effect. No effects were seen in mortality or in the physical observations, body weights and food consumption values, organ weights, neurobehavioral evaluations, terminal organ/body weights and macroscopic and microscopic evaluations.

1084 EFFECTS OF BIOCHANIN A ON IMMUNE FUNCTION AND CELL PROLIFERATION

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The effects of naturally occurring phytoestrogens on estrogen sensitive immune functions are not well understood. We examined the effects of the phytoestrogen, biochanin A (BA), *in vitro* on natural killer cell (NKC) activity as well as cell viability and proliferation of NKC sensitive (YAC) and NKC insensitive (EL4) cell lines. NKC activity was significantly enhanced in comparison to vehicle (VEH)(2.5% DMSO) treated preparations at BA molarities ranging from 10^{-6} to 10^{-18} M, however, this VEH concentration of DMSO significantly depressed NKC activity when compared to media alone. If the VEH concentration was reduced to 0.5% DMSO there was no significant difference between NKC activity in BA treated, VEH or media preparations. Neither BA ($5x10^{-6}$M) or VEH (0.5%DMSO) altered YAC or EL4 cell viability, however, BA dramatically inhibited cell growth in cultures examined at 20, 48, 72 and 144 hr of incubation. Acute *in vivo* doses of BA by gavage (8 mg/kg), or ip injection (4 mg/kg) did not alter NK activity when compared to VEH treatment. Additionally, multiple immune responses were assessed following a 3 week intermittent treatment by gavage or ip injection of BA or the respective vehicle. Eleven BA treatments were given during this period. IgG response to keyhole limpet hemocyanin immunization, delayed-type hypersensitivity (T cell response), and NKC activity as well as spleen and body weights were measured at the end of this period. We found no significant difference in any of these parameters with the exception of a significant increase in spleen wt in the ipBA treated rats (ipBA, 1.041 ± 0.027g; ipVeh, 0.902 ± 0.030g, $p<.01$). Our findings suggest that BA, like its secondary metabolite, genistein, inhibits cell proliferation. BA does not appear to have the negative effect reported for steroidal estrogen on immune functions such as NKC activity.

1085 THE SUBCHRONIC TOXICITY OF A PROTEASE CONCENTRATE IN RATS

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Historically, enzymes have been used in food preparation long before their actual "discovery." With the advent of modern enzymology, there has been a significant increase in the use of isolated and purified enzymes in foods and food processing procedures. Proteases, for example have been used in meat tenderization, baking and brewing. The subchronic oral toxicity of a protease concentrate was evaluated by incorporating it into the diet and feeding it ad libitum to three groups of Sprague-Dawley rats (20 animals/sex/group) for ninety consecutive days at dose levels of 0, 5000, 15000 and 50000 ppm. No signs of clinical toxicity were observed throughout the study. Similarly, no changes in hematology, clinical chemistry or urinalysis data were observed. However, gross necropsy revealed bilaterally pale and enlarged salivary glands in the 15000 and 50000 ppm dose groups for both males and females. Both absolute salivary gland weights and relative weight ratios were significantly increased above controls for the 50000 ppm group. Histopathological evaluation of the salivary glands revealed hypertrophy without degenerative or inflammatory changes. This sialoadenotrophic effect effect is believed to be a normal physiologic effect manifesting itself through existing neural reflex arcs. Based upon the results of this work, a NOAEL for this protease concentrate of 50000 ppm was recommended.

1086 ALKALINE TREATMENT OF AFLATOXIN B1: STABILITY OF PARENT AND DEGRADATION PRODUCTS

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Nixtamalization is the process whereby corn is treated in a solution of $Ca(OH)_2$ (pH > 12) thus aiding in the removal of the pericarp and production of flavor and texture characteristics associated with corn tortilla products. Previous work has demonstrated that this process also degrades aflatoxins by a proposed mechanism of hydrolysis of the lactone, thus reducing the levels of the toxins in nixtamalized corn (nixtamal) as analyzed by conventional methods. However, this chemical masking can be reversed to some degree upon acidification of the nixtamal. The purpose of this work was to: (i) ascertain the effects of time and temperature (within the parameters of nixtamalization) upon the degradation of aflatoxin B_1 (AfB_1); and (ii) determine the stability of AfB1 at various pHs. Results indicated that time and temperature caused no difference in the degradation of AfB_1. Breakdown of AfB_1 occurred at pH > 12 and reformation to AfB_1 occurred at pH < 4 with an observed hysteresis. An AfB_1 degradation product was isolated primarily in the aqueous layer between chloroform and H_2O of a 1ppm AfB_1-1% $Ca(OH)_2$ solution. High pressure liquid chromatography using photodiode array detection (PDA) of cold and ^{14}C-AfB_1 treated separately with $Ca(OH)_2$ and NaOH indicated the presence of an identical product more polar than the parent compound. The PDA detector indicated a hypsochromic shift at 310nm characteristic of an altered lactone moiety. This compound may be the previously proposed β-keto acid intermediate of AfB_1 degradation. Any modifications in the nixtamalization process should be made in light of these data to determine the fate of aflatoxin that may be present in the corn. (Supported by TAES H-6215 and ATP #999902-004)

1087 ISOTHERMAL ANALYSIS OF AFLATOXIN B, BINDING TO PHYLLOSILICATE CLAYS

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Previously, a phyllosilicate clay (HSCAS, or Novasil™) was shown to tightly bind aflatoxins and prevent aflatoxicosis in animals. Additional *in vitro* studies, through the use of ligands with functional groups in common with aflatoxin B_1 (AfB_1), indicated that the β-dicarbonyl (ketolactone) system may be involved in the mechanism of tight binding and selective chemisorption. In this study AfB_1 is isothermally (at 25°C) mixed with HSCAS and other sorbents. The amount bound (mol/kg) versus equilibrium concentration (mol/L) in the supernatant was graphed and compared to standard isothermal plots. It was found that the binding of AfB_1 to HSCAS fits the characterization of a L5 isotherm. An L5 isotherm has two plateaus suggesting at least two types of binding sites. The data was also fitted to multiple isotherm equations: Langmuir (L-Iso.), Freundlich (F-iso.), Langmuir-Freundlich combination (LF-iso.), Toth (T-iso.), and transforms of these equations. These equations were used to derive information and compare the strength of AfB_1 binding to a series of diverse aluminosilicates and to gain an insight into the molecular mechanism(s) and site(s) of AfB_1 adsorption. Information derived from the isotherms of AfB_1 and HSCAS include a capacity of 483 nmol/mg (LF-iso.), an energy distribution of binding centered at 35 KJ/mol (LF-iso.), a heterogeneity coefficient of 0.46 (T-iso.), and the distribution coefficients centered at 1.5×10^4, 3.5×10^4, and 6.6×10^4. The structures of AfB_1, various clays, and a proposed model for HSCAS were entered into a molecular modeling program to help understand the data derived from the various isotherms. Data obtained supports the hypothesis that AfB_1 may bind at two sites which could include the edges, within interlayers of HSCAS, and on

the basal surface of HSCAS particles (Supported by ATP 999902-004, NIH P42-ES04197, TAES H6215).

1088 EFFECTS OF CLAY AND CYCLOPIAZONIC ACID IN BROILER CHICKENS

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Cyclopiazonic acid (CPA) is a mycotoxin that has been found as a natural contaminant of corn and peanuts and reported to be produced by many strains of *Aspergillus flavus*, alone or in combination with the aflatoxins. Previous work to evaluate selected sorbents for binding CPA *in vitro* indicated that an acidic phyllosilicate (P^1) strongly adsorbed CPA from aqueous solution and also that low pH influenced binding of CPA to phyllosilicate clays. The objective of this study was to evaluate the efficacy of selected sorbents to protect against CPA toxicity in broiler chickens. Acidic phyllosilicate (P^1), a neutral phyllosilicate (P_2) and a zeolite (clinoptilolite) were evaluated for their ability prevent CPA toxicity in broiler chickens. Day-old chicks were fed diets containing 45 mg/kg CPA alone or in combination with 1% P_1, P_2 or clinoptilolite *ad libitum* from day 1 to day 21. Body weight, feed consumption, feed conversion, hematology, serum biochemical values and enzyme activities were evaluated. Compared to controls, CPA alone reduced body weight at day 21 by 26% and resulted in lower efficiency of feed utilization. CPA toxicity was also expressed through increased relative weights of kidney, proventriculus and gizzard. There were some alterations in hematology, serum biochemical values and enzyme activities. Clays were not effective in diminishing the growth inhibitory effects of CPA and the increased weights of organs. Although there was protection for some of the hematological, serum biochemical and enzymatic changes produced by CPA, test clays were apparently ineffective in preventing the major toxicity associated with CPA. Our results from this study suggest that the binding capacity and affinity of clay are critical for adsorption and efficacy of CPA *in vivo* (Supported by TAES H-6215 and ATP # 999902–004).

1089 DEGRADATION AND TOXICOLOGICAL EVALUATION OF FUMONISIN B_1 AND OTHER MYCOTOXINS TREATED WITH HYDROLYTICALLY-PRODUCED OZONE GAS

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Previous studies have demonstrated rapid degradation of the four naturally occurring aflatoxins by a novel method of ozone (O_3) production. The objectives of this study were threefold: 1) determine oxidative degradation of cyclopiazonic acid, fumonisin B_1 (FB_1), ochratoxin A, patulin, secalonic acid D and zearalenone, 2) evaluate the toxicity of ozonated FB_1, and 3) identify reaction products and propose a mechanism. Aqueous solutions of each mycotoxin were ozonated for 0, 15, 30 45 sec, 1.0, 2.5 and 5.0 min. Treatment for 15 sec with 10% wt O_3 (121mg/L) reduced equimolar concentrations of mycotoxins (i.e., 31.25 μM) to undetectable levels by high performance liquid chromatography. No new products were detected by absorbance or fluorescence, with the exception of FB, which yielded one new major OPA-reactive compound with a retention time of 9.8 min. this retention time did not correspond with parent FB_1 (10.3 min) or its hydrolyzed form (11.6 min). HPLC with electrospray MS detection of ozonated FB^1 solutions demonstrated the loss of two mass units, suggesting oxidation of the hydroxyl at C-3 forming 3-keto FB_1 (3-KFB_1). Despite parent FB_1 not being detected after 15 sec treatment, both FB_1 and solutions ozonated from 15 sec to 5.0 min caused an increase in the sphinganine/sphingosine ratio (Sa/So) ratio in liver slices thus providing further evidence of a compound with an intact primary amine. However, neither FB_1 or 3-KFB_1 were detected after 30 min treatment with 10% wt O_3. Additionally, a combination of O_3 with mass transfer enhancement and UV irradiation appeared to increase FB_1 and 3k-FB_1 degradation. (Supported by NIH P42-ES04917, USDA 95–33610–1429, and TAES H6215).

1090 ELEVATION OF SPHINGANINE IN LIVER SLICES BY EXTRACTS OF CORN NATURALLY CONTAMINATED WITH FUSARIUM MONILIFORME: SPECIFICITY OF THE RESPONSE TO FUMONISIN

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Fusarium moniliforme commonly contaminates corn, and produces the fumonisin mycotoxins. These toxins are the responsible agents that cause field intoxications in animals, including equine leucoencephalomalacia and porcine pulmonary edema. Fumonisins also cause liver and kidney toxicity in laboratory rodents, are tumor promoters, and may have cancer-initiating ability. The mode of action of the fumonisins is thought to be their ability to inhibit ceramide synthase, which catalyzes the conversion of sphinganine(Sa) and sphingosine (So) to ceramide. The result of this inhibition is disruption of sphingolipid biosynthesis, and accumulation of sphingoid bases, primarily Sa, in tissues. We previously showed that precision cut tissue slices can be used as a bioassay for fumonisin-like activity. Slices maintained in medium and exposed to fumonisins for short (12 to 48 hr) periods were found to have greatly elevated free Sa content. In this study we investigated whether acetonitrile:water (1:1) extracts of fumonisin-contaminated corn could elevate Sa in rat liver slices. The extracts significantly elevated Sa in the slices, even when dosed with extract equivalent to as little as 0.5 mg of corn. We also studied the specificity of the response by comparing hepatotoxins that act by differing mechanisms for their ability to elevate Sa. These toxins included aflatoxin B_1, beauvericin, carbon tetrachloride, cyclopiazonic acid, carbonyl cyanide, Sa, and fumonisin B_1. Slices were exposed to the toxins for 20 hrs, and then analyzed for free Sa content. Only fumonisin B_1 (FB_1) and Sa significantly elevated Sa. In control slices and slices dosed with toxins other than Sa or FB_1, free Sa content was <1.5 nmol/g tissue. Slices dosed with FB_1or Sa had 5–15 nmol/g tissue.

1091 THE INFLUENCE OF "FITIN" DRUG TO THE INDUCTION OF BRAIN AND LIVER MONOOXYGENASE SYSTEM

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It is known, that one of the main systems of organism is the monoorigenase system (MOS) of liver responsible for utilization of toxic products of metabolism. Majority of toxic substances entering in organism, are being exposed to oxidation in MOS of liver, brain, skin, lungs. Aim: Studying of concentration of paramagnetic heme-iron protein (HIP) and iron-sulfur protein (ISP) structures of liver and brain under influence of chronical intoxication and their dynamics during the treatment. Experimental investigations were conducted on 100 white rats of "Vistar" rase. Chronic intoxication was evoked by the way introduction of heliotrin alcaloid in dose 5mg./100g. of body mass, 1 time per week during 6 weeks. On the 60th day of experiment, we began to treat one part of animals, by fitin in dose 200 mg./kg. during 10 days. Killing of the animals was on 120th day of the experiment. Spectrum EPR of liver was being taken at temperature (−196 C) in vessel on device "SEPR-03". We determined the position of EPR signal (g-factor) and its amplitude in control and experimental samples. In spectrum of EPR typical signals from HIP of Cyt.P-450 with g-factors were being observed: g1 = 2.25, g2 = 2.42, g3 = 1.91 functioned in electron transport bonds of microsomes in the capacity of terminal oxidase, which plays main role in detoxication reactions. The signal with g-factor equal to 1.94 also was being determined connected with ISP in restored form localized in electron-transport bonds of mitochondria. Investigations showed that untreated rats had the expressed inhibition of Cyt.P-450. The reducing of index HIP (g = 2,25) to on 95% in liver and in brain; ISP (g = 1,94) on 72% was revealed. The treatment of rats by fitin leads to increasing of concentration of HIP paramagnit centres till standart, and level of ISP structures of liver and brain exceed the standart. The concentration of free radicals (g = 2,01) become less on 40% then at healthy rats. Correlation (r = 0,89) between the indexes of HIP and ISP in liver and brain was revealed. Therefore, fitin plays an important role in induction of MOS not only in liver, but and in brain. It is especially valuable for the raising of the role of hematoence-phalic barrier of brain at neurotoxical processes.

1092 HORMONALLY REGULATED EXPRESSION OF MOUSE FLAVIN-CONTAINING MONOOXYGEN-ASE (FMO) ISOZYMES

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The flavin-containing monooxygenases (FMOs) comprise a gene family of xenobiotic metabolizing enzymes responsible for the oxidation of nitrogen, sulfur, phosphorous, and selenium-containing compounds. Five forms of FMO have been identified and expression varies among species and tissue. Based on enzyme activity, protein, and mRNA levels, we have previously demonstrated the female-selective, female-specific, and gender-independent expression of FMO1, 3, and 5, respectively. In this study, gonadectomized mice were examined and the role of testosterone investigated to determine the hormonal modulation of FMO expression in the liver. Activity data indicates that upon castration, male hepatic levels increase; however, administration of testosterone to castrated animals returns activity to control levels. When female mice and ovariectomized mice were treated with testosterone, their hepatic levels were reduced to those of male mice. In addition to testosterone repression, results indicate a possible stimulatory role of female sex steroids. Isozyme protein and mRNA levels were also examined to gain insight into regulation of FMOs.

1093 CYSTEINE S-CONJUGATE OXIDATION BY FLAVIN-CONTAINING MONOOXYGENASES.

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Sulfoxidation may play a role in the nephrotoxicity/mutagenicity of some cysteine conjugates. In this study, we investigate the ability of rabbit liver microsomes to metabolize *S*-benzyl-*L*-cysteine (SBC), *S*-allyl-*L*-cysteine (SAC), *S*-(1,2-dichlorovinyl)-*L*-cysteine (DCVC), and *S*-(1,2,2-trichlorovinyl)-*L*-cysteine (TCVC) to their respective sulfoxides. HPLC methods were used to quantify products of enzymatic reactions. Incubation of 10 mM SBC, SAC, DCVC, and TCVC with rabbit liver microsomes at 37°, pH 7.4, produced time-, NADPH-, and protein-dependent product peaks that coeluted with synthetic standard sulfoxides. The specific activities were 4.2 ± 1.4, 2.8 ± 0.6, 0.10 ± 0.03, and 0.054 ± 0.017 nmol sulfoxide/ mg protein/ min for SBC, SAC, DCVC, and TCVC, respectively. To investigate the specific monooxygenases involved in these reactions, the effects of adding 1mM methimazole or benzylimidazole to microsomal incubations were examined. While oxidation of SBC and SAC did not decrease in the presence of benzylimidazole, these activities were almost completely inhibited by methimazole. DCVC activity decreased by ≈ 15% in the presence of benzylimidazole, but was not detectable in the presence of methimazole. TCVC activity was significantly decreased in the presence of both benzylimidazole and methimazole (≈75% and 90%, respectively). When the cysteine conjugates were incubated with cDNA-expressed rabbit flavin-containing monooxygenase (FMO) isoforms FMO1, FMO2, FMO3, and FMO5, no metabolites were detected in the presence of FMO2 or FMO5. FMO1 showed activity with SBC and SAC. All four compounds were active with FMO3 with relative activities SAC>SBC>DCVC>>TCVC. These results provide strong evidence that FMOs are the major enzymes responsible for SBC, SAC, and DCVC *S*-oxidations and are partially involved in TCVC oxidation. (Supported by NIH grant DK44295 and EPA graduate research fellowship).

1094 SIMULTANEOUS PURIFICATION OF TWO DISTINCT ISOZYMES OF THE FLAVIN-CONTAINING MONOOXYGENASE (FMO) FROM RAT LIVER

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Previously, we provided evidence that methionine (Met) was oxidized to methionine sulfoxide by rat liver FMOs. Kinetic studies of Met and *S*-benzyl-*L*-cysteine (SBC) sulfoxidations in the presence of methimazole suggested the involvement of different FMO isozymes. In this study, we have purified FMO1 and FMO3 simultaneously from rat liver by monitoring SBC and Met sulfoxidation, respectively. Solubilized microsomes were initially chromatographed on a Cibracron Blue 3A-agarose column and different peaks of *S*-oxidase activity were observed with Met and SBC. Fractions containing the highest SBC *S*-oxidase activity (corresponding to FMO1) were pooled and further purified using a Reactive Red 120-agarose column followed by an ADP-Sepharose column. Fractions containing the highest Met *S*-oxidase activity(corresponding to FMO3) were combined and further purified on a second Cibracron Blue column followed by an ADP-Sepharose column. FMO1 had a final purification of nearly 300-fold and FMO3 of 75-fold. The identity and purity of the proteins were confirmed by SDS-PAGE and Western blotting using antibodies raised against purified rabbit liver FMOs. Kinetics were determined with both purified preparations using methimazole, SBC, and Met as substrates. The V_{max}/K_m ratio of Met sulfoxidation by FMO3 was 2.9X greater than that obtained for FMO1. The V_{max}/K_m ratio of SBC sulfoxidation by FMO1 was 8.5X that obtained with FMO3. Kinetics with methimazole gave similar V_{max} values for both FMO1 and FMO3; however the K_m for FMO3 was nearly 5 times greater than for FMO1. (Supported by NIH grant DK44295).

1095 SULFOXIDATION OF THIOBENZAMIDE BY FLAVIN-CONTAINING MONOOXYGENASE IN THE ISOLATED PERFUSED RAT LIVER

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Hepatic microsomal flavin-containing monooxygenase (FMO) is known to catalyze the S-oxidation of thiobenzamide (TB) and to produce the thiobenzamide S-oxide (TBSO), a hepato-toxic metabolite whose fate in vivo is not known. Although several in vitro assay methods to determine the FMO activity are known, no in vivo method exists. Thus, an ex vivo method utilizing perfused rat liver is developed. As j dged by the release of hepatocellular lactate dehydrogenase into the perfusate, both the single pass and recycling perfusion of 1 mM TB did not produce any hepatotoxicity. The rate of TBSO production (1.2 to 1.4 nmoles /g liver /cycle) either by the single pass metabolism or by the recycling perfusion metabolism was not significantly different. This indicated that the re-reduction of TBSO back to TB is negligible and further showed that, at this rate of TBSO production, hepatotoxicity was not a critical factor for maintaining the FMO activity or the hepatocellular integrity in the isolated perfused liver for up to 50 min. Therefore, the developed FMO assay system utilizing recycling perfused rat liver may provide a useful tool for evaluation and for further assessment of the physiological role of FMO in intact animal.

1096 OXIDATIVE METABOLISM OF SELENO-L-METHION BY FLAVIN-CONTAINING MONOOXYGENASES

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Previously, we have reported that L-methionine is a substrate for flavin-containing monooxygenases (FMOs). Seleno-L-methionine (SM), a naturally occurring amino acid, a toxicant, and a potential anticancer agent, inhibited methionine sulfoxidation by FMOs. In this study, we examined the ability of SM to be metabolized by rabbit liver microsomes and by purified FMOs. SM selenoxide was synthesized by the reaction of SM with H_2O_2 and characterized by NMR and fast atom bombardment-MS. An HPLC method was developed to quantify enzymatically formed SM selenoxide after derivatization with 2,4-dinitrofluorobenzene. Formation of SM selenoxide in rabbit liver microsomes, was time-, protein concentration-, and NADPH-dependent. Inclusion of the P450 and peroxidase inhibitors, 1-benzylimidazole and KCN, or superoxide dismutase caused no inhibition of enzymatic activity. These results suggested the involvement of FMOs in SM metabolism. Further evidence for this hypothesis was provided by the findings that the kinetics of SM oxidation in rabbit liver microsomes, which revealed a high affinity K_m (0.09 mM) and a low affinity K_m (1.9 mM), were similar to those obtained with purified rat liver FMO3 (K_m = 0.25 mM) and FMO1 (K_m = 2.13 mM). Thus, FMOs may play a role in the biological activity of SM (Supported by NIH grant DK 44295).

1097 TISSUE-SPECIFIC ALTERATION OF EXPRESSION OF FLAVIN MONOOXYGENASE (FMO) ISOFORMS 1AND 2 BY DIETARY INDOLE-3-CARBINOL (I3C) IN MALE FISCHER 344 RATS

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FMOs, a multi-gene family of drug-metabolizing enzymes, catalyze the oxidation of many environmentally and pharmacologically important compounds. Despite the presence of FMOs in nearly all mammalian tissues, their endogenous function is unknown. FMO isoforms are both species- and tissue-specific, and developmentally regulated. FMOs are induced by steroid hormones,

but unlike other drug-metabolizing enzymes, they are quite refractory to modulation by dietary or other factors, and few, if any, exogenous inhibitors of FMO expression have been identified. Our lab has recently found that I3C, a tumor-modulating component of cruciferous vegetables that induces both phase I and phase II enzymes, is a potent inhibitor of FMO expression *in vivo*. Twenty-four male Fischer 344 rats, age 4 weeks, were acclimated for 7 days on AIN-76A powdered semipurified diet, then fed the same diet containing I3C (0–0.25%) for 7 additional days. On day 8 animals were killed by asphyxiation, and liver, lung, kidney and intestine were removed, frozen in liquid N_2, and stored at −80°C until analysis. FMO protein levels were immunoquantified by western blotting, and FMO activity was assayed by S-oxidation of substituted thiocarbamides. Presence of I3C metabolites in organs was verified by mass spectroscopy. In both lung and kidney no changes in FMO1 protein or activity levels were observed throughout the dosage range studied. In liver, however, FMO1 showed a 7-fold decrease in protein levels, and a 5-fold decrease in enzymatic activity. In intestine, results were even more dramatic, with almost total ablation of both protein levels and activity at the highest I3C dosage. No changes in FMO2 isoform levels were observed in any tissue at any dosage. This study indicates a possible independent regulatory mechanism for FMO isoforms 1 and 2. (Supported by HL38650).

1098 INITIAL ACTIVATION AND SUBSEQUENT INACTIVATION OF FLAVIN-CONTAINING MONOOXYGEANSE ACTIVITY IN RATS PRETREATED WITH METHIMAZOLE

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Unlike the P450 activity, the flavin-containing monooxygenase (FMO) activity is known not to be altered by pre-exposure to xenobiotics. In a preliminary experiment co-infusion of methimazole (MTZ), a well-known substrate and an inhibitor of the FMO, increased caffeine oxidation utilizing the perfused rat liver. Thus, to determine if the hepatic FMO activity is modulated in vivo by MTZ, rats were pretreated with MTZ (100 mg/kg, i.p.) at 4 hr before isolating the liver microsomes and the FMO activity was determined using N,N-dimethylaniline N-oxidation and thiobenzamide S-oxidation assays. FMO activity was increased by 20% but P450 activity by N_3-demethylation of caffeine and P450 contents were decreased significantly. Next, to determine the time course of this MTZ dependent modulation, they were measured in microsomes isolated at various times (2, 4, 6, 12, 24, and 72 hr) following the pretreatment with MTZ. FMO activity was maximally increased (20%) at 4–6 hr, returned to the normal level at 12–24 hr, and then suppressed by 30% at 72 hr. In contrast, P450 activity was suppressed continuously without the early stimulatory phase. This indicated that MTZ may destroy both FMO and P450, but during the period in which the MTZ sulfone produced by FMO is attached to the enzyme, it may protect FMO from MTZ dependent destruction. Thus, when in vitro FMO assay was carried out with MTZ-sulfone, it was stimulated initially at the low concentration of MTZ-sulfone. In support of this conclusion, MTZ-sulfone which is not produced by P450, did not protect the N_3-demethylation of caffeine catalyzed by P450 (P450 IA2) from the MTZ-destruction.

1099 ESTABLISHMENT OF FLAVIN-CONTAINING MONOOXYGENASES IN HEPATIC MICROSOMES OBTAINED FROM PRIMARY CULTURES OF RAT HEPATOCYTES

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Because bioactivation of many xenobiotics may result from the activity of various hepatic metabolic enzymes, such as cytochrome P-450 and flavin-containing monooxygenases (FMO), the need to establish the presence of the enzyme and its activity in *in vitro* hepatic systems is important for metabolic studies of various compounds. Presently, there have not been any reports for the presence of FMO in cultured rat hepatocytes. The objective of this study was to evaluate FMO found in microsomes of cultured rat hepatocytes grown with and without Matrigel® over a 3 day period. The activity of FMO was evaluated by following methimazole-dependent oxygen uptake polarographically with purified pig liver FMO and with cultured rat hepatic microsomes at 37°C in pyrophosphate buffer, pH 8.4, containing the glucose-6-phosphate NADPH-generating system. The results of these experiments demonstrated that there was FMO activity present in microsomes isolated from cultured rat hepatocytes over a 3 day period incubation. Furthermore, the presence of FMO in cultured rat hepatic microsomes was detected immunochemically using the Western Blotting technique. Thus, these results confirm the presence of FMO in our *in vitro* primary culture system of rat hepatocytes. These results will allow for further investigations of metabolism of xenobiotics through the FMO pathway in our primary culture system of rat hepatocytes. (We acknowledge Dr. D M Ziegler for supplying the purified FMO and the antibody. This work was supported by NIDDK grant#1 F31 DK09063–01).

1100 TISSUE CONCENTRATIONS OF BUTADIENE EPOXIDES IN RODENTS FOLLOWING MULTIPLE AND SINGLE EXPOSURES TO 1,3-BUTADIENE (BD) BY INHALATION

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Rats and mice metabolize inhaled BD to the mutagenic butadiene monoepoxide (BDO) and highly mutagenic butadiene diepoxide (BDO_2). Higher tissue levels of BDO and BDO_2 were attained in male mice than in male rats during a single, low-level exposure to BD. In the present study, these metabolites were measured in tissues of female Sprague-Dawley rats and $B6C3F_1$ mice after either a single 6-h exposure or a 6-h exposure daily for 10 days by nose-only inhalation to a concentration of 62.5 ppm. Tissues were taken at the end of the exposure(s); volatile metabolites were isolated by cryogenic vacuum distillation and quantified using isotope dilution multidimensional gas chromatography/mass spectrometry. BDO levels in blood, femurs and lungs of both species did not differ significantly between the two exposure regimens. After repeated compared to single exposures, BDO levels were 5- and 1.6-fold higher in mammary tissue and 2- and 1.4-fold higher in fat tissue in rats and mice, respectively. BDO_2 levels in rats were 7.7 ± 2.2 and 12.5 ± 0.8 pmol/g in fat and in mice were 265 ± 19 and 191 ± 29 pmol/g in mammary tissue after single and multiple exposures, respectively. There was ~ 15% less of this metabolite in the fat of mice after repeated exposures compared to a single exposure. The finding that BDO - itself a mutagen and the precursor of highly mutagenic BDO_2 - accumulates in rodent fat may be important in assessing the potential risk to humans from inhalation of BD. [Research sponsored by the Chemical Manufacturers Association under Funds-in-Agreement No. DE-FIOY-91AL66351 with the U.S. DOE under Contract No. DE-ACO4-76EV01013.]

1101 DETOXICATION OF DIEPOXYBUTANE BY HYDROLYSIS AND GLUTATHIONE CONJUGATION IN RAT, MOUSE, AND HUMAN LIVER IN VITRO

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Butadiene (BD) is carcinogenic in rodents, with mice being more susceptible than rats. Metabolism of BD to epoxides, particularly 1:2-epoxy-3-butene (BMO) and 1:2, 3:4-diepoxybutane (BDE), is thought to play a key role in BD carcinogenicity. Significant species differences exist in the *in vitro* activation of BD to BMO and BDE with mice >> rats ≈ humans. A quantitative understanding of the balance between formation and detoxication of BDE is essential to improve assessment of human risk following exposure to BD. The purpose of this study was to assess whether species difference exist in the detoxication of BDE. We investigated the hydrolysis of BDE and the conjugation of BDE with glutathione (GSH) in subcellular fractions prepared from liver of $B6C3F_1$ mice, Sprague Dawley rats, and humans. Hydrolysis and GSH conjugation of BDE followed Michaelis-Menten kinetics in human, rat, and mouse liver subcellular fractions. The rate of hydrolysis was mouse < rat < human. In microsomes prepared from human liver samples (n = 6), K_M averaged 5.0 pm 0.6 (SE) mM and V_{max} 158 pm 31 nmol/mg protein/min. The rate of GSH conjugation was human < rat sg mouse. In human cytosol (n = 6), K_M averaged 2.1 pm 1.4 mM and V_{max} 6.4 pm 1.6 nmol/mg protein/min. These results indicate that there is a large difference in contribution between the different routes of detoxication of BDE among species. GSH conjugation plays a predominant role in mice, while hydrolysis is by far the most important detoxication pathway in humans.

1102 OXIDATION OF 3-BUTENE-1,2-DIOL BY ALCOHOL DEHYDROGENASE

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3-Butene-1,2-diol (BDD) is a secondary metabolite of 1,3-butadiene. The metabolic fate of BDD is unknown. A possible pathway for metabolism of BDD is oxidative dehydrogenation, catalyzed by alcohol dehydrogenase (ADH). In this report, oxidation of BDD by horse liver ADH (HLADH) and rat liver cytosol are characterized. Enzymatic oxidation of BDD was followed spectrophotometrically, by measuring formation of NADH. At high substrate concentrations, both R- and S-enantiomers of BDD were rapidly oxidized by HLADH. However, the rate of oxidation of S-BDD was much higher than that of the R-enantiomer. Oxidation of BDD by HLADH was inhibited by 4-methyl pyrazole (4-MP). BDD was also oxidized by rat liver cytosol, and the reaction was inhibited by 4-MP. Extracts of incubation mixtures containing racemic BDD and either HLADH or chloroperoxidase (a bacterial enzyme known to oxidize primary alcohols to aldehydes) were examined by gas chromatography for the presence of reaction products. A new GC peak (retention time 2.7 min) which was dependent on the presence of substrate, cofactors, and enzyme was detected. The peak was strongly attenuated in the presence of 4-MP or glutathione, and has been identified by GC/mass spectrometry as 2-hydroxy-3-butenal in chloroperoxidase extracts. Identification was based on the presence of the expected molecular ion (*m/z* 86) and the presence of two other ions (*m/z* 29 and *m/z* 57) expected from fragmentation of 2-hydroxy-3-butenal. These data demonstrate oxidation of 3-butene-1,2-diol by mammalian ADH, and suggest formation of the aldehyde 2-hydroxy-3-butenal, a potentially toxic species. (Supported by NIH ES06841)

1103 DISCOVERY OF NOVEL AND BIOLOGICALLY ACTIVE ARACHIDONIC ACID METABOLITES

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Arachidonic acid (AA) is the precursor to various bioactive compounds. P-450 epoxygenases can epoxidize AA to form four bioactive regioisomers of *cis*-monoepoxyeicosatrienoic acid. AA monoepoxides are hydrolyzed to the corresponding *vic*-dihydroxyeicosatrienoic acids or DHETs by soluble epoxide hydrolase (sEH). These *vic*-diols are bioactive, also. One long-term interest of our laboratory has been EH metabolism of fatty acid diepoxides. Recently, we demonstrated mouse liver microsomal and S-9 conversion of polyunsaturated fatty acids to their diepoxides and diepoxide hydrolysis products (THF-diols). Hence, we hypothesized that AA diepoxides and THF-diols existed, *in vivo*. We tested our hypothesis by chemical extraction of mouse liver and a subsequent analysis of the extracted lipids using GC/NICI/MS which led us to the discovery of AA THF-diols, *in vivo*. Further work demonstrated that these metabolites can exist in free acid as well as acylated forms in many tissues and that their production is inducible. Our *in vitro* studies have shown that synthetic mixtures of AA THF-diols can rapidly elevate intracellular calcium levels in isolated pulmonary epithelial cells. Mixture of the precursor AA diepoxide elevated intracellular calcium levels at a significantly lower rate. After purifying the mixture of AA THF-diol isomers we observed that some of the regio-and positional isomers of AA THF-diols were more potent than others and some were inactive in the intracellular calcium mobilization assay. Because AA THF-diols are bioactive and their biogenesis is inducible, these novel metabolites should be viewed as important chemical mediators able to alter cellular homeostasis.

1104 METABOLISM OF 4-VINYLCYCLOHEXENE AND VINYLCYCLOHEXENE 1,2-MONOEPOXIDE TO VINYLCYCLOHEXENE DIEPOXIDE IN B6C3F$_1$ MICE: AUTOINDUCTION BY VCH

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Since bioactivation of 4-vinylcyclohexene (VCH) to vinylcyclohexene diepoxide (VCD) is critical for VCH-induced ovotoxicity, experiments were initiated to demonstrate the formation of VCD *in vivo* and *in vitro* in mice. Circulating levels of VCD were observed between 0.25 and 2 hr following administration of a single dose of vinylcyclohexene 1,2-monoepoxide (1,2-VCHE; 2.74 mmol/kg, ip). Following administration of a single dose of VCH (7.5 mmol/kg, ip), only 1,2-VCHE was detected in blood between 1 and 4 hr. When multiple doses of VCH (7.5 mmol/kg/d × 10 d, ip) were administered, both 1,2-VCHE and VCD were detected in blood. 1,2-VCHE levels were elevated 2-fold following multiple doses of VCH versus a single dose at all time points (1–3 hr) examined. Elevated epoxide levels were not due to an accumulation of these metabolites, as they were not detected in the blood 24 hours after the last dose. The rate of epoxidation of 1,2-VCHE to VCD was also increased in hepatic microsomes from VCH-treated mice (4.6 nmol/min/mg), as compared to hepatic microsomes from control animals (2.1 nmol/min/mg). Similar increases in microsomal conversion of 1,2-VCHE to VCD were observed following induction with acetone (2 ×), phenobarbital (3.6 ×), and dexamethasone (2.8 ×). VCH treatment produced an increase in total P450 of 30%. Substantial elevation of immunoreactive isoforms of P450 including 2E1 and members of the 1A, 2A, and 2B subfamilies was detected. Thus, a critical process for VCH-induced ovotoxicity is autoinduction of its metabolism, which involves multiple P450 isoforms. (Supported by the Center for Toxicology/Flinn Research Fellowship, Chemical Manufacturers Association, NRSA #F32-ES05613, and Center Grant #P30-ES06694).

1105 METABOLISM AND BIOACTIVATION OF ACRYLONITRILE (ACN) BY LIPOXYGENASE

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Based on laboratory studies, there is scientific evidence that high doses of ACN cause cancer in rats. The potential carcinogenic risk to humans from ACN exposure is uncertain. Since inhalation is the primary route of ACN exposure in humans, it is important to understand its metabolism in human lung. Here, we report linoleic acid dependent co-oxidation of ACN catalyzed by lipoxygenase partially purified by Concanavalin A affinity chromatography from human lung cytosol (HLLO). Highly purified soybean lipoxygenase (SLO), extensively used as a model lipoxygenase, was also employed for comparison. Lipoxygenase converted ACN to a metabolite which reacted with p-nitrobenzyl pyridine to yield a purple adduct exhibiting a spectral peak at 560 nm suggesting the formation of 2-cyanoethylene oxide (CEO). ACN metabolism was dependent on the concentration of LA, ACN and the enzyme at pH 7.4. The reaction was linear with time for approximately 30 min. In the absence of the enzyme or in the presence of boiled enzyme, there was no appreciable ACN metabolism. Using [^{14}C]ACN, significant covalent binding to protein and calf thymus DNA was observed in the presence of both SLO and HLLO suggesting the generation of electrophilic ACN intermediates. The binding to both protein and DNA was dependent on the enzyme concentration and time. The enzymatic ACN epoxidation and covalent binding to protein and DNA were inhibited by nordihydroguaiaretic acid (lipoxygenase inhibitor) and by antioxidants BHA and BHT. These results strongly suggest that lipoxygenase is capable of ACN metabolism and bioactivation to reactive species.

1106 THE EFFECTS OF WATER RESTRICTION ON THE ACTIVITY OF DRUG METABOLIZING ENZYMES IN VITRO IN THE RAT

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Water deprivation has been shown to impair the in vivo metabolism of antipyrine and lidocaine. In the present study, the effect of water restriction on drug metabolizing enzymes in the rat, was investigated. 3 groups of adult SD male rats (n = 6) were maintained in metabolic cages during the water restriction period of 10 days. Control rats (C) had water ad lib. The second and third groups received 60% (40-WR) and 20% (80-WR), respectively of the water consumed by C rats. All rats had food ad lib. At the end of the water restriction period rats were sacrificed. The activities of aniline hydroxylase (AH), EROD, glutathione-S-transferase (GST) with chlorodinitrobenzene as a substrate and glucuronyl transferase with p-nitrophenol as a substrate in hepatic fractions were assayed by spectrophotometric or fluorimetric methods. No difference was observed in the enzymatic activities of the above mentioned enzymes between 40-WR rats and C rats. GSH concentration was 10% lower in the 80-WR rats as compared to C rats (5.68 ± 0.27 vs 6.30 ± 0.16 umol/g liver). The activity of GST was 25% lower in the 80-WR compared to C rats (0.93 ± 0.05 vs 1.25 ± 0.06 umol/min/mg prot, respectively). The activity of EROD was 3 times higher in the 80-WR rats as compared to control rats (0.117 ± 0.014 vs 0.08 ± 0.006 nmol/min/mg prot, respectively). There was no difference in the activity of AH and glucuronyl transferase between 80-WR rats and C rats (0.583 ± 0.082 vs

0.574 ± 0.049ug/min/mg prot and 15.258 ± 1.341 vs 15.652 ± 0.612 pmol/min/mg prot, respectively). These results show that only severe water restriction of 80% affected hepatic drug metabolizing enzymes. It is difficult to extrapolate to in vivo situation but changes in certain enzyme activities such as EROD may increase the toxicity of certain drugs and environmental pollutants.

1107 *IN VITRO* METABOLISM OF THE COMUTAGEN 2-AMINO-3-METHYLPYRIDINE

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2-Amino-3-methylpyridine (2A3MP) is known to be mutagenic in the presence of norharman and a metabolic activating system (S9) (Sugimura et al., 1982). In order to investigate the mechanism of comutagenesis we have examined the metabolism of 2A3MP using fortified hepatic fractions (microsomal and S9) from the rat and rabbit. 2A3MP was metabolised by both species to yield 2-amino-3-methylpyridine N-oxide (2A3MPNO), 2-amino-3-hydroxymethyl-pyridine (2A3HMP) and 2-amino-3-methyl-5-hydroxypyridine (2A3M5HP) which were identified by comparison of their chromatographic (HPLC) and spectral properties (UV) with those of authentic compounds. Metabolic products having the characteristics of 2-hydroxylamino-3-methylpyridine (2H3MP) or the corresponding nitroso (2NO3MP) or nitro (2N3MP) compounds could not be conclusively identified. The mutagenicity of 2A3MP, 2N3MP, 2H3MP and 2A3MPNO towards Salmonella Typhimurium TA98 in the presence and absence of norharman and presence or absence of S9 was investigated. The results showed that 2A3MPNO was not mutagenic whereas 2A3MP, 2N3MP and 2H3MP were highly mutagenic in the presence of norharman and S9, with 2H3MP being 5 times more active than 2N3MP and 40 times more potent than the parent amine. The results obtained support the concept that comutagenicity of aminoazaheterocycles is associated with oxidation products of the exo amino group rather than nuclear N-oxidation. Sugimura T., Nagao M., and Wakabayashi K. (1982) Adv. Exp. Med. Biol. 136B, 1101–1125.

1108 CONTINUOUS TREATMENT WITH DICHLOROACETATE IN DRINKING WATER ALTERS ITS METABOLISM IN RATS BUT NOT IN MICE

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Dichloroacetate (DCA) is one of the haloacetates found in drinking water as by-product of the chlorinating process that assures the potability of water. It is also a potent hepatocarcinogen in rats and mice. Studies in humans have indicated that sequential dosing with DCA inhibits its clearance from blood. These changes are frequently associated with alteration in the metabolism that can have implications for chronic toxicity. The present study was undertaken to determine whether such effects can be reproduced in rodents. Male F344 rats and B6C3F1 mice were pretreated with DCA in drinking water at 2 g/L for 14 days. The fifteenth day the rodents were administered ^{14}C-DCA i.v. at 5, 20, 100 mg/kg. Metabolites were recovered from expired air, urine and feces for 24 hr. Clearance of DCA was examined in control and pretreated animals with the 100 mg/kg dose. Pretreatment curtailed the metabolic conversion of DCA to CO_2 in rats but not in mice. The initial metabolic rate of the high dose in rats was reduced from 11.55 mg equiv. of DCA kg^{-1} hr^{-1} to 0.72 and to a somewhat lesser extent at the lower doses. The terminal half-life was increased from 2.52 to 10.97 hr. and the amount of DCA eliminated unchanged in urine was increased by a factor of 12. These data suggest that one pathway to CO_2 is virtually eliminated by concentrations of DCA that have been previously shown capable of inducing hepatic tumors in rodents. What remains is a low affinity pathway that appears to be largely saturated for up to 24 hours at doses as low 20 mg/kg. These data suggest that rat pretreatment with DCA does inhibit its metabolism, similar behavior to that previously reported in humans (This work was supported by AWWARF/NWRI Contracts 701-92 /699-516-92).

1109 INHIBITION OF PULMONARY PENTOXYRESORUFIN O-DEALKYLASE ACTIVITY PROTECTS AGAINST BUTYLATED HYDROXYTOLUENE BUT NOT NAPHTHALENE-INDUCED LUNG TOXICITY

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The lung injury and decrease in pulmonary calpain activity normally caused by the administration of butylated hydroxytoluene (BHT), in BALB/c mice, was prevented by pre-treatment with O,O,O-trimethylphosphorothioate. This pretreatment, which inhibited pulmonary pentoxyresorufin O-dealkylase (PROD) activity, also prevented the outward signs of poisoning with naphthalene but afforded no protection against injury to the lung. Naphthalene-induced injury did not result in any decrease in pulmonary calpain activity. In contrast, pretreatment with pyrazole, which induced pulmonary PROD activity, exacerbated both the lung injury and the decrease in pulmonary calpain activity caused by BHT, but it did not modify naphthalene-induced lung injury. Pulmonary PROD activity was closely correlated with the activation of BHT but a reduction of this activity, to less than 10% of the normal level, did not prevent naphthalene-induced lung injury. The isoenzyme responsible for naphthalene activation is, therefore, unlikely to be involved in the activation of BHT or to constitute a significant component of lung PROD activity in mice.

1110 N-ACETYLATION OF THE HETEROCYCLIC AMINE, BATRACYLIN, BY MOUSE, RAT, AND HUMAN LIVER

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Batracylin (BAT; 8-aminoisoindolo[1,2-b]quinazolin-12(10H)-one), a heterocyclic amine, exhibits species variations in its toxicological effects which are associated with formation of acetylbatracylin (ABAT). Acetylation of heterocyclic amines is catalyzed by N-acetyltransferases (NAT), designated as NAT1 and NAT2. Individuals are classified as rapid or slow acetylators based on activity of NAT2. The acetylation of BAT was compared to activities of known substrates of NAT in mouse, rat, and human hepatic cytosol. BAT acetylation in F-344 rat cytosol (5.32 ± 0.16 nmol ABAT/min/mg) was six fold greater than that of mice (C-57B1/6J and A/J). BAT acetylation in rapid acetylators (C-57B1/6J) was similar to that in slow acetylators (A/J) while there was a two-fold difference in acetylation of 2-aminofluorene (2-AF). Nine human liver samples were evaluated using sulfamethazine (SMZ), a preferential substrate for human NAT2, p-aminobenzoic acid (PABA), a preferential substrate for NAT1, and BAT. The BAT NAT activities ranged from 14.9 to 0.56 nmol ABAT/min/mg. N-Acetylation of BAT in rapid acetylators was similar to activity observed in rats, while activity in slow acetylators was similar to that of mice. The Km in rapid acetylators for BAT, SMZ, and PABA was 6.59 ± 3.21, 277.5 ± 69.4, and 31.21 ± 12.5 μM, respectively, which did not significantly differ from slow acetylators. However a significant difference in Vmax for BAT and SMZ was observed between phenotypes. Variability in Vmax was observed for PABA but the differences were independent of phenotype. A significant correlation (r^2 = 0.97, $p < 0.001$) was observed between the intrinsic clearance (Vmax/Km) of BAT and SMZ. These data demonstrate that BAT is a preferential substrate for human NAT2. (Supported by ES-05174 and NIEHS Center Grant IP30 ES06694)

1111 BILIARY EXCRETION OF 2,4-D AND METABOLITES IN MALE AND FEMALE RATS, MICE AND HAMSTERS

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2,4-Dichlorophenoxyacetic acid (2,4-D) is a widely used broadleaf herbicide and one of several compounds being used in a National Toxicology Program study of peroxisomal proliferators. As part of this study the role of the biliary system in the elimination of 2,4-D was evaluated in male and female Sprague-Dawley rats, B6C3F1 mice and Syrian hamsters. Following cannulation of the bile duct under pentobarbital anesthesia, an intragastric dose of ^{14}C-2, 4-D (200 mg/kg and 5 μCi) was administered and bile was collected at 30 (rats) or 60 (mice and hamsters) minute intervals for 4-6 hours. Bile flow was constant in rats, increased in mice and decreased in hamsters throughout the collection periods. Total recovery of radioactivity was greatest in male mice (about 6% of administered dose over 4 hours). Female mice and rats of both sexes excreted about 3% over the same interval and male and female hamsters about 1%. About 71–88% of the activity in bile was parent com-

pound. The glycine conjugate of 2,4-D was found in bile from all three species and both sexes. The only sex-dependent difference in the metabolite profile was in mice. Male mice excreted twice as much glycine conjugate as female mice. Two additional unidentified metabolites were present in rat urine and one of these was detected in mouse urine along with the taurine conjugate. This study shows that there are significant species differences in the biliary elimination and metabolism of 2,4-D but few sex-dependent differences.

1112 BIOTRANSFORMATION OF NNK, A TOBACCO-SPECIFIC NITROSAMINE IN THE ISOLATED PERFUSED RAT LUNG

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The rodent lung is a target organ for NNK carcinogenicity. In the present study, we examined the metabolism of the chemical in the isolated perfused rat lung. The lung, in an anesthetized male Sprague-Dawley rat, was ventilated in situ, excised, and perfused in a constant temperature (37 °C) chamber with 10 uM [^{3}H]NNK in bovine serum albumin-supplemented Krebs-Ringer phosphate buffer, and aliquots of the perfusate analyzed at different time points for metabolites. NNK metabolism was linear for at least 60 min, during which 20% of the compound was metabolized via pyridine N-oxidation, alpha-hydroxylation and carbonyl reduction. The major metabolites identified in other metabolic systems, NNAL and NNK-N-oxide, were also the major metabolites in the perfused rat lung and were formed in a 1:1 ratio. Hydrolysis of the perfusate with beta-glucuronidase prior to analysis showed that only NNAL was conjugated, with the conjugate representing 20% of the total NNK formed. The data show that the intact lung, a target tissue, possess all of the NNK biotransformation pathways described in other tissues. Supported by ES06414 and ES05022.

1113 A NOVEL MECHANISM OF LIPOXYGENASE MEDIATED THIOETHER FORMATION FROM ETHACRYNIC ACID

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Ethacrynic acid (EA), a diuretic drug is known to interact with glutathione s-transferases in the presence of reduced glutathione (GSH) to yield a EA-GSH conjugate. Here we propose a new mechanism for the formation of EA-GSH conjugate in a lipoxygenase (LO) mediated reaction involving the oxidation of GSH to a GS radical. Similar to the glutathione s-transferase mediated reaction, EA-GSH conjugate generated by LO exhibited an absorbance maximum at 270 nm. The conjugate formation was dependent on the concentration of linoleic acid, EA, GSH, and LO. The optimal conditions to observe maximum rate of conjugate formation (~258 nmoles/min/nmole enzyme) included the presence of 0.4 mM linoleic acid, 1 mM GSH, 50 nM LO, and 0.2 mM EA at pH 9.0. Classical LO inhibitors e.g. NDGA, gossypol and ETI significantly inhibited EA-GSH conjugation. The LO generated EA-GSH conjugates were isolated by high pressure liquid chromatography and quantified radiomatically using labelled GSH. These results suggest that LO, which is primarily known for xenobiotic bioactivation, may represent yet another important pathway for GSH conjugate formation that could lead to potential detoxification of chemicals.

1114 METABOLISM AND DISPOSITION OF [^{14}C-] CYCLOHEXENE OXIDE IN MALE FISCHER 344 RATS FOLLOWING ORAL ADMINISTRATION

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Cyclohexene oxide (CHO) is a monomer used in the synthesis of pesticides, pharmaceuticals, perfumery and dyestuffs. Since little is known about its disposition, [^{14}C]-CHO (10 and 100 mg/kg) was administered orally to male F-344 rats. Blood samples were obtained at various time points throughout the experiment and analyzed for parent compound and metabolites using GC equipped with a flame ionization detector (FID). Unaltered CHO was not detected in the plasma at the time points examined (15 min - 48 hr). However, cyclohexanediol was detected in the plasma 15 min following oral administration of [^{14}C]-CHO. The majority of the radioactivity (75–90%) was eliminated in the urine within 48 hr. Approximately 8% of the total radioactivity was detected in the feces and less than 2% was excreted as either [^{14}C]-CO_2 or volatile organic compounds. In the urine, about 10% of the dose was eliminated as cyclohexanediol and 40% as the cyclohexanediol glucuronide conjugate. HPLC analysis of urine samples showed additional metabolite peaks which were suspected to be other glucuronide and mercapturic acid conjugates. Mass spectra obtained were consistent with the glucuronide conjugate of cyclohexanediol and N-acetyl-S-(2-hydroxycyclohexyl)-L-cysteine. (Supported by NIEHS, NO1-ES-35367 and Southwest Environmental Health Sciences Center Grant P30 ES06694-01)

1115 TESTOSTERONE AND BOLDENONE METABOLITES GENERATED BY DOG LIVER MICROSOMES: SYNTHESIS AND ISOLATION

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Microsomal enzymes contribute to the biotransformation of anabolic steroids as demonstrated in previous studies. Metabolites produced contain a significant amount of androgenic activity. The quantitative production of anabolic steroid metabolites in a liver microsome system was investigated. To evaluate the metabolism of anabolic steroids, microsomes from male beagle livers were incubated with testosterone or boldenone and cofactors for 24 hours. By 20 hours, the rate of steroid depletion remained constant at 28 ng/mL/min and 16 ng/mL/min for testosterone and boldenone, respectively. This method generated 16 testosterone and 14 boldenone metabolites. These metabolites were separated on a reverse-phase HPLC system utilizing a 250 x 4.6 mm C_8 analytical column interfaced to an on-line ultraviolet spectral detector. Each metabolite generated a characteristic wavelength maximum at 240 nm. This system efficiently resolved the numerous stereoisomers within an elution time of 10 minutes. Metabolite molecular weights were determined by HPLC/MS. In order to extract and collect larger quantities of metabolites from microsomal supernatant, a solid-phase extraction system was developed. Steroids from eluting fractions were initially detected by an enzyme-linked immunosorbent assay followed by HPLC confirmation. I_{50} values were determined for most metabolites. This quantitative method was successful in steroid separation and quantitation.

1116 MEASUREMENT OF 2-THIOTHIAZOLIDINE-4-CARBOXYLIC ACID (TTCA) AS AN INDEX OF THE ABILITY OF DITHIOCARBAMATES TO RELEASE CARBON DISULFIDE IN VIVO

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Dithiocarbamates are a class of compounds used in agriculture, industry and medicine. Subchronic to chronic exposures to some dithiocarbamates have resulted in the development of neuropathy in humans and experimental animals. Under physiological conditions, dithiocarbamates readily decompose to their parent amine and CS_2, suggesting that the neurotoxicity of dithiocarbamates may be mediated through release of CS_2. Previous investigations have demonstrated *in vitro* CS_2-mediated covalent crosslinking of proteins by *N, N*-diethyldithiocarbamate (DEDC) and *in vivo* covalent crosslinking of erythrocyte spectrin for CS_2. In this study, rats, housed individually in metabolic cages, were given daily equimolar *i.p.* or oral doses of DEDC, disulfiram, *N*-methyldithiocarbamate (NMDC) or CS_2 for 5 days. The relative ability of these dithiocarbamates to release CS_2 *in vivo* was assessed by measuring urinary TTCA, a metabolite of CS_2 used as a biomarker of internal exposure to CS_2. TTCA was detected in all of the treated animals beginning 24 hours after the initial exposure. For both routes of administration, the relative quantity of TTCA eliminated in urine for each compound was disulfiram > DEDC ≃ CS_2 > NMDC. The amount of TTCA eliminated in urine following oral administration was greater than that from *i.p.* administration for each compound tested. In a second experiment, oral administration of DEDC to rats 3 times/week for 7 weeks resulted in covalent crosslinking of erythrocyte spectrin These results demonstrate the ability of dithiocarbamates to release CS_2 *in vivo* and suggest that the greatest bioavailability of CS_2 occurs with dialkyldithiocarbamates and oral administration. This is especially relevant to disulfiram which is given orally over long periods of time for treatment of alcoholism and has resulted in the development of a neuropathy.

1117 EFFECTS OF BENZENE AND UNLEADED GASOLINE COEXPOSURES ON BENZENE METABOLISM AND TOXICITY

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Benzene (BZ) is present in unleaded gasoline (UG) as a small fraction of the liquid volume. Several components of UG are substrates for the same cytochrome P450 isozyme, 2E1, that catalyzes the oxidation of BZ. Since BZ toxicity most likely results from its oxidative metabolism, the toxicity of BZ as a component of UG may be decreased relative to the toxicity of BZ alone. The present study was performed to determine the effects of UG and BZ coexposures on BZ metabolism and genotoxicity in male B6C3F$_1$ mice. In metabolism studies, mice were exposed by nose-only inhalation for 6 hr to ^{14}C-BZ alone, fully-vaporized UG (FUG) and ^{14}C-BZ, or light-fraction UG (LUG) and ^{14}C-BZ. Coexposures with UG and BZ altered the profile of BZ metabolites recovered in urine over 48 hr. In mice exposed to BZ alone, phenyl sulfate (PS), hydroquinone glucuronide (HG) and muconaldehyde (MUC) accounted for approximately 90% of urinary metabolites. Pre-phenylmercapturic acid (pre-PMA), phenyl glucuronide (PG) and phenol were minor metabolites. In mice exposed to either FUG or LUG with BZ, PS alone accounted for 90% of the urinary metabolites. Formation of HG and MUC was greatly reduced; more pre-PMA was excreted than either HG or MUC. To correlate genotoxicity with changes in BZ metabolism, micronuclei (MN) formation was quantitated. When compared to air-exposed controls, significant increases in MN formation were observed in polychromatic erythrocytes (PCE) from bone marrow of mice exposed to BZ alone ($p \leq 0.01$) and mice exposed to FUG with BZ ($p \leq 0.01$). No increase in MN formation was detected in PCE from bone marrow of mice exposed to LUG with BZ compared to air-exposed controls. These results suggest that the inhibitive effect of LUG on BZ metabolism decreases BZ genotoxicity. FUG also inhibits BZ metabolism. However, coexposure to FUG and BZ results in genotoxicity, possibly due to one or more of the greater than 300 hydrocarbons present in FUG. (This work was supported in part by the American Petroleum Institute.)

1118 METABOLIC FATE OF PROFLAVINE AND ACRIFLAVINE IN RAINBOW TROUT

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Proflavine and acriflavine (10-methyl-proflavine) are potentially useful as antiinfectives in fish. We characterized their metabolic fate to better understand their pharmacokinetics and total residue depletion. Rainbow trout at 12 °C were administered proflavine (10 mg/kg) or acriflavine (6 mg/kg) by intraarterial injection. At 16 hr (proflavine) and 5 hr (acriflavine) after administration, three metabolites for proflavine and two metabolites for acriflavine were observed using HPLC. They were isolated from plasma and liver using a cyanopropyl solid phase extraction column. Acid hydrolysis showed that the metabolites were conjugates of proflavine and acriflavine. Hydrolysis with β-glucuronidase in 0.1 M phosphate buffer (pH 6.8) at 37 °C for 24 hr indicated that two metabolites of each parent were glucuronides. β-Glucuronidase activity was totally inhibited by 20 mM saccharic acid 1, 4-lactone. Matrix-assisted, laser-desorption, time-of-flight mass spectrometry verified that the proflavine conjugates were N-acetyl-, N-glucuronosyl-, and N-acetyl-N'- glucuronosyl-proflavine conjugates. The conjugates of acriflavine were N-glucuronosyl- and N-acetyl-N'-glucuronosyl-acriflavine. Finally, metabolite structures were also confirmed by synthesis. (Supported by FDA contract number 223-93-7014).

1119 COMPARATIVE METABOLISM AND EXCRETION OF [^{14}C]TOLUENE IN FISCHER 344 RATS AND B6C3F1 MICE

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Toluene (TOL) is a high volume commodity chemical used extensively as a solvent, a chemical intermediate, and in many applications as a replacement for benzene. The metabolism of TOL in rats at oral dose levels at or above 100 mg/kg has been studied; however, at lower dose levels, reflective of potential human exposure, information is not available. The objective of these studies was to extend the range of doses studied, and to provide comparative data on metabolism and excretion at much lower dose levels than previously studied. Excreta were collected for 48 h from rats and mice that had been given oral doses of [^{14}C]TOL at levels of 0.005–50 mg/kg. Total radioactivity in urine, feces, and exhaled breath was quantified by liquid scintillation spectrometry (LSS) and individual metabolites were quantified by HPLC/LSS. In rats, >90% of all doses were excreted in the urine and <1% in the feces within 48 h. Rat urinary metabolites consisted of hippuric acid (HA, >95% of [^{14}C]TOL urinary metabolites), S-benzyl mercapturate (ca. 2% of [^{14}C]TOL urinary metabolites), and trace quantities of benzoic acid (BA). Some volatile organics (ca. 7% of dose) were exhaled in breath in the highest dose studied, however <1% of the dose was exhaled in breath at the lower dose levels. While HA accounted for 50–70% of the urinary metabolites in mice, BA and at least two other metabolites not seen in rat urine were also present. One of these (15–20% of [^{14}C]TOL urinary metabolites) has been tentatively identified as o-cresol-O-glucuronide based on its enzymatic hydrolysis to o-cresol. The other metabolite (ca. 3–8% of [^{14}C]TOL urinary metabolites) has not yet been identified. Although only minor dose-dependent changes in metabolism were found within a species, based on these studies, there would appear to be quantitative differences in TOL metabolism between rats and mice.

1120 INVESTIGATION OF COVALENT BINDING OF REACTIVE METABOLITES OF PENTACHLOROPHENOL BASED UPON LIVER PROTEIN ADDUCTS IN SPRAGUE-DAWLEY RATS AND B6C3F1 MICE

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Reactions of eletrophilic metabolites of pentachlorophenol (PCP), i.e., tetrachloro-1,2-benzoquinone (Cl_4-1,2-BQ), tetrachloro-1,4-benzoquinone (Cl_4-1,4-BQ), tetrachloro-1,2-benzosemiquinone (Cl_4-1,2-SQ) and tetrachloro-1,4-benzosemiquinone (Cl_4-1,4-SQ), with sulfhydryl groups of liver proteins, were investigated *in vivo* in male Sprague-Dawley rats and B6C3F1 mice. Liver cytosolic and nuclear proteins were isolated 24 h after a single oral dosage of pentachlorophenol (0-40 mg/kg body weight). Production of cysteinyl adducts of quinones and semiquinones in liver proteins was proportional to the dosage of PCP administered. Least-squares regression analysis of adduct concentration versus dosage suggests species differences in the formation of semiquinone-and quinone-derived protein adducts in the liver. The rate of formation of cysteinyl adducts of Cl_4-1,2-BQ in mice was at rates 3 fold greater than that of Cl_4-1,4-BQ, while no cysteinyl adducts of Cl_4-1,2-BQ were detected in rats, suggesting that the dominant quinones produced in mice and rats are Cl_4-1,2-BQ and Cl_4-1,4-BQ, respectively. Relatively small amounts of cysteinyl adducts of semiquinones were produced in mice when compared to those of rats. Additionally, the production of multi-S-substituted adducts of Cl_4-1,4-BQ in nuclear proteins of mice was 2-10 fold greater than rats, suggesting the formation of extensive protein-protein crosslinks in mouse liver nuclei. The results of this study provide a basis for the estimation of the tissue dose of PCP-derived quinones from *in vivo* metabolism.
This work is supported by the National Institute of Environmental Health Sciences grant P42ES05948.

1121 DIFFERENCES IN METABOLISM OF OCHRATOXIN A AND B BY RAT LIVER MICROSOMES

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The mycotoxin ochratoxin A (OTA), consisting of a 5'-chloro-3,4-dihydro3-methylisocoumarin moiety linked by an amide bond to L-phenylalanine is produced by some species of the genera *Aspergillus* and *Penicillium*. The occurrence of OTA in food and feed is widespread and it is known to be nephrotoxic and carcinogenic to several animal species. Ochratoxin B (OTB), the corresponding dechlorinated analogue, is also produced by the fungi to a lesser extent and is much less toxic. We have observed that 4-hydroxylation of both OTA and OTB is greatly induced (~40-fold) in microsomes from 3MC pretreated rats; however, the pH optimum for OTA metabolism is ~6.0 while that for OTB metabolism is ~7.0. This difference appears to be related to the pKa$_1$ of the phenolic hydroxyl of OTA (~7.0) and OTB (~8.0). This influences the binding of substrate to P450 1A1 and its subsequent metabolism.
(Supported by the Kidney Foundation of Canada)

1122 CYSTATHIONASE γ-LYASE CONTRIBUTES TO THE DETOXIFICATION OF CYANIDE IN VIVO

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While the results of several *in vitro* studies have suggested that the enzyme cystathionase γ-lyase (E.C. 4.4.1.1) may function in the endogenous detoxification of cyanide, this possibility has not been investigated *in vivo*. If cystathionase γ-lyase is involved in the endogenous detoxification of cyanide, it logically follows that inhibiting cystathionase γ-lyase should increase the toxicity of cyanide. To test the hypothesis that cystathionase γ-lyase may be involved in the detoxification of cyanide, the activity of cystathionase γ-lyase was inhibited *in vivo* with a suicide inhibitor of cystathionase γ-lyase, propargylglycine. The activity of liver cystathionase γ-lyase activity was decreased 96.8% by administration of propargylglycine, indicating that the propargylglycine treatment was effective. The propargylglycine treatment did not affect the activity of thiosulfate: cyanide sulfurtransferase (EC 2.8.1.1, rhodanese) or 3-mercaptopyruvate:cyanide sulfurtransferase (EC 2.8.1.2), two other enzymes which have been suggested to be involved in the detoxification of cyanide. In rats treated with propargylglycine the LD_{50} of cyanide was 5.14 ± 0.029 mg/kg, which was significantly lower than the 5.98 ± 0.008 mg/kg LD_{50} of cyanide in control rats. The results of these studies suggest that cystathionase γ-lyase does participate in the detoxification of cyanide *in vivo*.

1123 THE METABOLISM OF n-NONANE IN MALE FISCHER 344 RATS

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The urinary metabolites of n-nonane in male Fischer 344 rats, after administering the hydrocarbon by gavage, included γ-valerolactone, δ-hexanolactone, 2, 5-hexanedione, δ-heptanolactone, 1-heptanol, 2-nonanol, 3-nonanol, 4-nonanol, 4-nonanone and 5-methyl-2-(3-oxobutyl)furan. Metabolism strongly favored the formation of monoalcohols and lactones, which are the products of appropriately substituted hydroxy carboxylic acids. The metabolites were identified using gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). High pressure liquid chromatography (HPLC) permitted the detection of the dicarboxylic acids malonic acid and glutaric acid in the n-nonane dosed rat urines.

1124 EXPRESSION OF ELECTROPHILE-INDUCIBLE GENES IN CYCLOPHOSPHAMIDE-SENSITIVE AND RESISTANT MCF-7 HUMAN BREAST CANCER CELLS

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Developed tolerance of cancer cells to anticancer drugs frequently limits effective chemotherapy, which often is dependent on the formation of electrophiles (Ep). For example, tumor cell killing by cyclophosphamide (CPA) is dependent upon its metabolism to the potent DNA-alkylating agents phosphoramide mustard and acrolein. However, these reactive intermediates upregulate Ep-inducible genes via an Ep response element (EpRE); these Ep-upregulated enzymes serve to diminish the susceptibility of the cancer cell to long-term anticancer drug exposure by enhancing breakdown and elimination of reactive intermediates. The human breast cancer mutant cell line MCF-7/HC is much more resistant to 4-hydroxycyclophosphamide (4H-CPA) than parent MCF-7 cells. Part of the difference is due to higher levels of aldehyde dehydrogenase and glutathione S-transferase in the mutant cells. Herein we report that MCF-7/HC cells express elevated enzyme activities and mRNA levels of NAD(P)H:menadione oxidoreductase (NMO1), aldehyde dehydrogenase (ALDH3c) and γ-glutamylcysteine synthetase (GCS), as well as increased GSH levels. These findings suggest that GSH and elevated expression of Ep-inducible genes may be responsible for the genetically-programmed resistance of MCF-7/HC cells to CPA. (NIH, T32-ES07278, P30-ES06096 and RO1-AG09235)

1125 TOXICOLOGICAL FOUNDATIONS OF ECOLOGICAL RISK ASSESSMENT.

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During the 1980s, ecological risk assessment had played a secondary role to human health concerns. In 1990, it was recommended in a report produced by the EPA's Science Advisory Board on the appropriate priorities and future strategies that as much importance be attached to reducing ecological risk as human health risk. Since then ecological risk assessment methodology has developed rapidly, and it is now the primary focus of the EPA's Risk Assessment Forum to prepare the very first guidelines for ecological risk assessment. This workshop will demonstrate current, state-of-the-art ecological risk assessment methods. Emphasis will be placed on the use of toxicological information in the ecological risk assessment and risk management process. Both field and laboratory studies that can enhance these processes will be described. The significance of the proposed guidelines and their potential impact upon present and future-ecological risk assessment methods will be discussed.

1126 INFLAMMATORY MEDIATORS OF HEPATOTOXICITY

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Gram negative bacterial sepsis is associated with multiorgan failure and high mortality in patients. The host reponse to the lipopolysaccharide (LPS) component from bacterial cell walls can account for much of the organ injury observed during severe sepsis. In addition, exposure to quite small doses of LPS markedly enhances the toxic response to xenobiotic agents, raising the possibility that the degree of systemic exposure to this ubiquitous agent may be a determinant of individual susceptibility to other toxicants.

Injury to the liver that results from LPS exposure occurs through the actions of numerous cellular and soluble mediators of inflammation. For example, studies in rats have implicated the neutrophil as a critical mediator of LPS hepatotoxicity, and results from coculture studies with hepatocytes in vitro suggest that neutrophil-derived proteases play an important role in hepatocellular killing. An activated coagulation system is also required for the expression of LPS-induced liver injury, and results of studies in vivo and in isolated livers point to thrombin as a critical mediator that acts distally on cells in a network of events that culminate in liver injury. Understanding cellular interactions and mechanisms of LPS-induced injury may lead to effective interventions for important clinical problems and also increase our understanding of individual variation in toxic responses to xenobiotic agents.

1127 MITOCHONDRIAL-MEDICATED CELL INJURY

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Mitochondria are central to cellular energy metabolism, redox control, osmotic regulation, pH balance and cytosolic Ca^{2+} homeostasis. Thus, chemical-induced mitochondrial dysfunction can have diverse effects on extramitochondrial functions, and considerable effort is often required to distinguish between toxic mechanisms occurring directly through mitochondrial targets and those in which mitochondrial dysfunction only plays a modulatory role in cell death caused by damage to extracellular targets. Mitochondria are generally susceptible to damage from oxidants and electrophiles as well as lipophilic cations and lipophilic weak acids. Of particular interest, the mitochondria contain a small genome which is essential for mitochondrial function and not well protected against DNA damage. Consequently, accumulated mitochondrial DNA damage can contribute to chronic toxicity even if short-term studies do not reveal mitochondrial dysfunction. Mitochondrial dysfunction is often organ-specific because the mitochondrial phenotype is cell specific with regard to enzyme and tranporter content, subcellular localization and mitochondrial volume density within cells. Variations in mitochondrial content and characteristics are also known to occur with chronic hypoxia, aging, and other pathophysiologic conditions so that risk of toxicity due to agents affecting mitochondria is likely to vary considerably in association with other health-related parameters.

1128 DISRUPTION OF RETINAL MITOCHONDRIAL FUNCTION BY FORMATE IN METHANOL TOXICITY

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Formate, the toxic metabolite in methanol poisoning, has been hypothesized to produce retinal and optic nerve toxicity by disrupting mitochondrial energy production. *In vitro* studies in our laboratory have shown that formate inhibits the activity of cytochrome oxidase (C.O.), reduces mitochondrial ATP synthesis and inhibits mitochondrial glutamate oxidation in rat and bovine retinal preparations. We have also investigated the actions of formate on mitochondrial function *in vivo* utilizing a rodent model of methanol-intoxication (Eells, 1991). Methanol-intoxicated rats developed formic acidemia, metabolic acidosis and retinal dysfunction analogous to human intoxication. Morphologic studies coupled with C.O. histochemistry revealed photoreceptor mitochondrial disruption and a profound reduction in retinal C.O. activity in methanol-intoxicated rats compared to control animals. Biochemical measurements also showed a significant reduction in retinal C.O. activity and retinal ATP concentrations in methanol-intoxicated rats relative to control animals. No differences from control values were observed in hepatic, renal or cardiac C.O. activity or ATP concentrations in methanol-intoxicated rats. Selective inhibition of mitochondrial function in the retina is consistent with both the functional and morphologic findings in methanol intoxication and supports the hypothesis that formate acts as a retinal mitochondrial toxin in methanol poisoning. (Supported by The American Petroleum Institute and NIH grants RO1-ES06648 and P30-EYO1931).

1129 HEPATOCYTE MITOCHONDRIAL BIOENERGETICS AS AFFECTED BY PHENYL HERBICIDES

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Herbicides of the main chemical families have been assayed for hepatocyte toxicity and mitochondrial bioenergetic alterations. Dinoseb acts as a powerful uncoupler at nM range. 2,4-D partially inhibits the redox chain and uncouples at mM range. Paraquat dissociates oxidative phosphorylation through lipid peroxidation and partially inhibits the redox chain and ATP synthesis. These effects might certainly contribute for the observed toxicities in isolated hepatocytes. Dinoseb, as expected, discharges the energetic load of hepatocytes and the ratio GSH/GSSG with good correlations to loss of cell viability ($r^2 = 0.988$ and 0.972). 2,4-D depresses GSH/GSSG and increases cell Ca^{++} with correlations to loss of cell viability of $r^2 = 0.99$. Paraquat significantly decreases GSH/GSSG and the energetic load in close correlations with loss of viability ($r^2 = 0.981$ and 0.992). These effects are putatively primary events associated with cell death and citotoxicity promoted by these herbicide compounds widely used in agriculture programs. Supported by JNICT.

1130 QUINONE-INDUCED INTERFERENCE WITH MITOCHONDRIAL CALCIUM REGULATION

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A number of structurally diverse chemical compounds are known to interfere with the transport of calcium across the inner mitochondrial membrane. The result is a loss of ion and volume regulation, membrane depolarization, and inhibition of oxidative phosphorylation. Successful rescue with fructose or inhibitors of the mitochondrial calcium transporters or the permeability pore implicate the mitochondrion as an important intracellular target in the manifestation of chemical-induced cell injury. The molecular mechanism by which xenobiotics alter mitochondrial calcium regulation depends on the chemical reactivity of the substance in question. Oxidants or redox cycling quinones alter the voltage sensor of the mitochondrial permeability pore resulting in an increased sensitivity to calcium-induced membrane depolarization and inhibition of respiration, which are prevented by inhibitors of the permeability transition. Electrophilic quinones, on-the-other-hand, covalently arylate critical nucleophiles associated with the electron transport chain resulting in a calcium-independent depolarization of the mitochondrial membrane. Inhibitors of the permeability transition pore are not effective in protecting cells against electrophilic quinones. Accordingly, although mitochondrial dysfunction is implicated in the cytotoxicity of numerous agents, there exists distinct mechanisms by which this is manifested, depending on the chemical reactivity of the specific xenobiotic in question.

1131 MITOCHONDRIAL HEAT SHOCK PROTEINS RECOGNIZE DEHYDROGENASES INACTIVATED BY REACTIVE CHEMICAL METABOLITES

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Mitochondria are the major energy producing organelle. Most mitochondrial proteins are products of nuclear genes and are imported. Heat shock proteins (Hsps), or molecular chaperones, play a vital role in mitochondrial biogenesis by facilitating the import, targeting and proper folding of polypeptides. Our data suggest that Hsps and other mitochondrial proteins are targets for toxicants. Cysteine conjugates, such as S-(1,1,2,2-tetrafluroethyl)-L-cysteine (TFEC) are potent mitochondrial toxicants. The oxoketoglutarate dehydrogenase complex (OGDC) is inactivated by TFEC-derived reactive metabolites. In kidneys from TFEC treated rats, the E2o and E3o components of OGDC contain difluorothioamidyl-lysine adducts. These adducted forms of E2o and E3o form stable complexes with mitochondrial Hsp60. When rat kidney mitochondria are incubated *in vitro* with [^{35}S]TFEC, ^{35}S-label is also found on these proteins. Immunoprecipitation studies suggest that Hsp60 preferentially binds [^{35}S]DFTAL containing proteins. However, Hsp60 itself is also a target for TFEC-derived reactive metabolites. Adducted Hsp60 may not associate with other adducted proteins. Thus, Hsp60 recognizes mitochondrial protein damage and is a target for reactive intermediates which damage mitochondrial function.

1132 A DICHOTOMY OF PROTECTION BY BCL-2 FROM MITOCHONDRIAL POISONS

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Bcl-2 is a mitochondrial protein that represses programmed cell death; its anti-apoptic activity could be the result of (1) a direct antioxidant role or (2) because Bcl-2 provokes an antioxidant response. We find that cells overexpressing Bcl-2 are protected from death induced by the mitochondrial oxidants H2O2, tBOOH, BSO, doxorubicin and xanthine/xanthine oxidase. However Bcl-2 did not protect cells treated with the respiratory chain inhibitors MPP+, rotenone, cyanide and antimycin A. Thus while mitochondrial oxidants trigger programmed cell death, acute poisons of the electron transport chain kill cells by an independent mechanism. SOD activity is elevated in Bcl2-overexpressing cells, and the amount of deletion mutations in mitochondrial DNA is lower. These three sets of observations are consistent with model 2, i.e. Bcl-2's protective effect works through induction of antioxidant protection.

1133 NOVEL AFLATOXIN-LYSINE SPECIFIC MONOCLONAL ANTIBODIES FOR BIOMARKER STUDIES

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Exposure of aflatoxin B_1 (AFB), a naturally occurring mycotoxin and a known human carcinogen (Group I), is one of the major risk factors in the etiology of human hepatocellular carcinoma (HCC). The development and application of highly sensitive and specific methods for detecting AFB and associated biomarkers is critical for identifying individuals at high risk. In this study, a mouse monoclonal antibody (IIA4B3) was developed against a synthetic AFB-lysine-BSA conjugate. The isotype of IIA4B3 has been characterized as IgG1λ. The affinity and specificity of IIA4B3 were further characterized and compared to a currently employed monoclonal antibody (2B11) by competitive radioimmunoassay (RIA). The affinity of IIA4B3 to AFB and associated biomarkers are ranked in order: AFB-lysine > AFB-FAPyr > AFB = AFB-N^7-Guanine > AFM_1 > AFQ_1. IIA4B3 had about 10-fold higher specificity to bind to AFB-lysine adduct than AFB when using ^{3}H-AFB-lysine as the tracer. The concentration of 50% inhibition for AFB-lysine is 680 fmol, compared to 7.0 pmol for AFB. IIA4B3 had about 7-fold and 2-fold higher affinity than 2B11 to recognize AFB-N^7-Guanine and AFB-FAPyr adducts when using ^{3}H-AFB as the tracer. In contrast, 2B11 had about equal affinity to recognize AFB, AFB-lysine, and AFM_1, followed by AFB-FAPyr, AFB-N^7-Guanine, and AFQ_1. An analytical method based on competitive RIA using IIA4B3 and ^{3}H-AFB-lysine has been established with the limit

of detection at 10 fmol and has been applied to measure AFB-albumin adduct levels from human serum samples collected from the residents of areas with high risk of AFB exposure and HCC. (Supported by NIEHS grant P01ES06052 and P30ES03819)

1134 PORPHYRIN PROFILE COMPONENTS AND SUB-CHRONIC 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN RESPONSIVENESS: A MULTI-SPECIES ANALYSIS

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Baseline hepatic porphyrin profile components from wildlife and laboratory species were compared and contrasted. Samples were analyzed from fish, amphibians, reptiles, birds and mammals. Relative abundance of specific porphyrins are, in order of decreasing concentration: 4-, 2-, 8-, 7-, 6- and 5- carboxylporphyrin. Specific concentrations of porphyrins, however, vary between species. As polychlorinated dibenzo-p-dioxins have been shown to inhibit heme biosynthetic enzymes and alter porphyrin profiles, dosing studies were performed with TCDD in chickens (*Gallus gallus*), B6C3F1 mice, deer mice (*Peromyscus maniculatus*), and alligators (*Alligator mississippiensis*). Total liver porphyrins were elevated over controls by 1.6 and 38 times in chickens and B6C3F1 mice, respectively. Elevation of 8-, 7-, and 4-carboxylporphyrins were responsible for chicken total porphyrin increases. B6C3F1 mice had significant increases in 8- and 7- carboxylporphyrins only. These data suggest that the porphyrin profiles were similarly susceptible to TCDD exposure in the animal species tested and that porphyrin profiles are an applicable biomarker of contaminant exposure for many diverse species. Sponsored by NIEHS ES04696 and USFWS.

1135 BIOCHEMICAL AND MORPHOLOGICAL EFFECTS OF AROCLOR - 1254, DICHLOROGUAIACOL AND GUAIACOL IN RAINBOW TROUT

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Anthropogenic waste effluents containing a variety of organic chemicals are often discharged into aquatic environments. The aim of this study was to assess the systemic effects of subclinical doses of Aroclor-1254 (AR), 4,5-dichloroguaiacol, and guaiacol in rainbow trout (*Oncorhynchus mykiss*). Fish were intraperitoneally exposed to the test chemicals in corn oil at dosage rates of 0 (control), 5, 10 and 25 mg/kg. At 96 hours postexposure, the fish were sacrificed and monitored for systemic biochemical and histological changes. Fish treated with AR showed a dose dependent increase in (i) the activity of aryl hydrocarbon hydroxylase and fatty degeneration in liver, and (ii) interrenal tissue proliferation in the anterior (hematopoietic) kidney. A marked (80–120%) increase in 7-ethoxycoumarin-O-deethylase activity was also observed in the livers of fish treated with a high dose (25 mg/kg) of these chemicals. At the dosages used, these chemicals did not cause any significant change in (i) liver and kidney succinate oxidase activity under phosphorylating and nonphosphorylating mitochondrial states, and (ii) plasma levels of thyroxin, lactic acid, total protein, lactate dehydrogenase and aspartate aminotransferase. These types of changes indicate that low level exposure to these chemicals can induce early systemic effects but does not manifest other cytotoxic lesions (mitochondrial dysfunction/release of cytoplasmic enzymes).

1136 IMPACT OF ENVIRONMENTAL POLLUTION ON CARBOXYLESTERASES IN THE GAR FISH FROM THE MISSISSIPPI RIVER BASIN

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The objective of this study was to evaluate, by means of biomarkers, the effects of environmental contaminants on the spotted gar fish (*lepisosteus oculatus*) from two sites, Devil's Swamp (DS) an industrial site known to be highly contaminated with chlorinated hydrocarbons and heavy metals, and Tunica swamp a non-industrial site. Carboxylesterases (CaEs), acetylcholinesterase (AChE) and butyrylcholinesterase were investigated in the gar fish. Significantly lower AChE, BuChe and CaE activities were observed in the microsomal and cytosolic fractions of brain and liver of gars from the contaminated site. The inhibition of esterase activities ranged from 23% to 47%. Greatest decrease in esterase activity was observed with the CaE activity in the liver. In *in vitro* studies, several chemicals, HCBD, Cd, Hg, and Pb, typical of DS contaminants, were found to inhibit brain AChE and liver CaE activities. High resolution isoelectric focusing of liver microsomal and cytosolic CaEs of gars was also studied. Activity assay of sliced gels of liver samples from both sites showed a major esterase peak with a pI of 4.89, with the peak significantly lower in the samples obtained from DS. Funded by a grant from DOE.

1137 NATURAL KILLER CELL FUNCTION IN RODENTS EXPOSED TO 60 HZ MAGNETIC FIELDS

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Increased NK cell activity has been reported in neonatal F344 rats exposed for 6 weeks to 60 Hz magnetic fields (Mandeville *et al.*, Proc. Bioelectromagnetic Soc., 132, 1994). Although statistical significance was attained only in rats exposed to 20 G fields, linear increases in NK cell function were reported throughout the field strength range of 20 mG to 20 G. Given the importance of NK cell activity in host defense, the present study was designed to evaluate the influence of subchronic exposure to 60 Hz magnetic fields on NK cell function in adult F344 rats and B6C3F1 mice. Animals were exposed continuously to linearly polarized pure sinusoidal 60 Hz fields of 10 G, 2 G, 20 mG, or 0 G (sham control), or intermittently (1 hr on, 1 hr off) to 10 G fields. Positive controls consisted of a single iv injection of antiasialo GM1 antibody. After 6 and 13 weeks of exposure, 10 animals per sex per group were euthanized for assessment of NK cell activity, in a standard 4 hr chromium release assay. NK cell function was not consistently affected by up to 13 weeks of magnetic field exposure in either in male B6C3F1 mice or male or female F344 rats. Although NK activity tended toward suppression in female mice following 13 weeks of exposure, no consistent statistically significant dose effect was observed. These results suggest that exposure to 60 Hz magnetic fields does not adversely affect NK cell function under the exposure conditions utilized in this study. (Supported by contract NO1-ES-25351 from the National Toxicology Program, National Institute of Environmental Health Sciences)

1138 BIOMONITORING USING POCKET GOPHERS AT ROCKY MOUNTAIN ARSENAL: EFFECTS OF DIELDRIN

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Burrowing animals may be ideal wildlife sentinels of exposure to soil-borne toxicants. The Plains pocket gopher (*Geomys bursarius*) is abundant at Rocky Mountain Arsenal where high soil levels of dieldrin persist. This study incorporates biochemical endpoints potentially responsive to chronic dieldrin exposure: cytochrome P-450 activity, liver glutathione content, brain biogenic amines, histopathologic changes, and tissue organochlorine pesticide residues. Soil pesticide residues from burrowing activities of individual pocket gophers are also measured. In laboratory-housed Plains pocket gophers, the maximum tolerated dose of dieldrin for 5 daily oral doses was 24 mg/kg/d in males and 16 mg/kg/d in females. Significantly elevated liver/body weight ratios were observed at these doses, as well as in males given 4.8 mg/kg/d. If biochemical markers are identified that provide more sensitive detection of dieldrin-related effects, the pocket gopher may serve as a valuable biomonitoring species at RMA.

1139 CLINICAL EFFECTS AND LESIONS IN LIVESTOCK FOLLOWING EXPOSURE TO A MULTIPHASE SOUR CONDENSATE/GAS PIPELINE LEAK

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The health of 500 head of beef cows plus calves was investigated as a monitor of environmental quality following a pipeline leak occurring under an iced-over river. Cattle within 3 miles of the leak were evaluated for health and productivity. The cattle were exposed to crude sour petroleum, emissions from burning the sour condensate from river ice, and emissions from steam washing of river gravel and aeration of river and wash water. Findings were irritation of mucous membranes, abnormal sexual behavior, and decreased bonding of cows with newborn calves, and failure of calves to thrive. Some

cows had proprioceptive-locomotor deficits. The clinical findings were supported by lesions in the respiratory, immune and nervous systems of exposed cattle. Respiratory tract lesions included hyperplasia of submucosal mucous gland, perivascular-nodular lymphoid accumulations, and loss of cilia. There was decreased lymphoid follicular development in lymphoid tissues of neonatal calves. Neuropathology included medulloblastoma, cytoplasmic vacuolation of neurons in the Gasserian ganglia, and axonal neuropathy of peripheral nerve fibers of the trigeminal nerve.

1140 CHARACTERIZATION OF HEMOGLOBIN (Hb) TRINITROBENZENE ADDUCTS

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1,3,5-trinitrobenzene (TNB) is used primarily in explosive compositions and munitions and has been detected as an environmental contaminant of surface waters as well as ground waters near production waste disposal sites. Hemoglobin (Hb) adducts of various environmental compounds have recently been proposed as biological markers of exposure assessment. In the present study, we investigated the formation of trinitrobenzene Hb adducts. We evaluated three potential binding sites (β-SH cysteine, N-terminal valine and α-aspartate residues) in four different species (shrew, vole, peromyscus and CD-1 mice) for the formation of TNB-Hb-adducts. Animals were administered orally with a single dose of [cf11]14C]-TNB (50 mg/kg bwt; 2.5 mCi/kg bwt) and the blood was collected after 24, 48 or 72 hrs. After separation of the RBC, a volume of the lyzate was used to precipitate the globin while aliquots of the lyzate were analyzed by HPLC and the resulting fractions collected for counting. The total adducts concentration as well as the concentration of the Hb adducts formed at the α and β chains of Hb, were found to be similar for the four species. The total binding of the [^{14}C]-TNB to hemoglobin was found to range from 1 to 3 pmol/mgHb. Although approximately 50% hemoglobin associated radioactivity was released during sample preparation, the binding of TNB to the α chain was found to be 4 times higher than the binding to the β chain suggesting that amino acids other than cysteine may play a role in adduct formation. These results indicate that Hb adducts of nitrobenzenes and related compounds may be suitable biomarkers of exposure assessment. (This abstract does not necessarily reflect USEPA Policy).

1141 EFFECT OF BURROWING BEHAVIOR ON THE ACCUMULATION OF SEDIMENT ASSOCIATED PHENANTHRENE BY MARINE AMPHIPODS

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The bioaccumulation of the Polycyclic Aromatic Hydrocarbon (PAH) phenanthrene by three different amphipod species was studied to investigate the extent to which benthic marine amphipods can modulate exposure to sediment associated contaminants. In this series of experiments, three infaunal marine amphipod species; *Eohaustorius estuarius* (non tube-forming, burrowing amphipod), *Leptocheirus plumulosus* (burrow-building amphipod) and *Grandidierella japonica* (tube-building amphipod), were exposed to ^{14}C-phenanthrene under two experimental conditions. 1) Sediment spiked at a concentration resulting in an interstitial water concentration of 2.5 ug/l phenanthrene with clean overlying water. 2) Sediment spiked at a concentration resulting in interstitial water concentration of 2.5 ug/l and the overlying water spiked at 2.5 ug/l phenanthrene. The bioaccumulation of phenanthrene was followed over 72 hours. In all three species of amphipods, the accumulation of phenanthrene was significantly greater in the spiked sediment and spiked overlying water exposure than the spiked sediment only exposure. There was a three to ten-fold difference in the tissue residue between treatments at 72 hours depending on species. The tube and burrow building species were able to decrease exposure to phenanthrene to a greater extent than the free burrowing species. The results suggest that behavioral differences among benthic species may be an important factor in determining exposure to sediment pollutants and that standard partitioning relationships may not accurately predict the bioaccumulation of PAHs from sediment. (This research supported by NIEHS ES07060 and Hatfield Marine Science Center's Markham Research Award).

1142 CHARACTERIZATION OF CHANNEL CATFISH GLUTATHIONE S-TRANSFERASES

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The glutathione *S*-transferases (GSTs) play a key role in protecting against environmental carcinogens and epoxide intermediates. In the current study, we examined GST expression in channel catfish, a common freshwater aquatic toxicological model. Channel catfish hepatic cytosolic fractions exhibited GST activity toward (±)-*anti*-benzo[a]pyrene-trans-7,8-dihydrodiol-9,10-epoxide (BPDE), and toward four prototypical GST reference substrates. Detectable GST activity toward aflatoxin B_1-8,9-epoxide (AFBO) was not observed. Western blotting studies revealed moderate immunological cross-reactivity of catfish GSTs to polyclonal antibodies raised against rat α and π class GST isozymes, and limited immunological cross-reactivity to rat μ class polyclonal antibodies. GST isozymes were purified to electrophoretic homogeneity from channel catfish hepatic cytosol by affinity chromatography and chromatofocusing. Three major and one minor GST isozymes were identified. All GST isozymes isolated exhibited catalytic activity toward BPDE. Interestingly, the specific GST-BPDE activity of one isozyme was more than 7-fold that of the other isozymes. These results indicate that the GST-BPDE activity in channel catfish liver cytosol is apparently due to the presence of multiple GST isozymes. Furthermore, these studies suggest that channel catfish GSTs share some structural and functional similarities with rodent GSTs.

1143 ELEMENTAL ANALYSIS IN CETACEANS STRANDED ALONG THE TEXAS GULF COAST

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Due to their long life span, cetaceans can be used as bioindicators of persistent toxins, such as metals in the marine environment. An extensive study analyzing numerous inorganic elements in a large population of marine mammals has not previously been performed. We determined the concentrations of 15 elements in 5 tissue types (liver, kidney, muscle, blubber, and bone) from cetaceans stranded along the Texas Gulf coast in the years 1991 to 1995. The metals analyzed include chromium, iron, manganese, cobalt, nickel, copper, zinc, arsenic, selenium, rubidium, strontium, molybdenum, cadmium, mercury, and lead. The primary technique used for elemental analysis was inductively coupled plasma - mass spectrometry (ICP-MS). In addition, unusual levels were confirmed by cold vapor absorption and flame atomic absorption or graphite furnace atomic absorption spectrophotometry, depending upon the element. We found variable, but occasionally high levels of iron, zinc, selenium, mercury and lead in the various tissues. Some of these levels were higher in the Gulf of Mexico animals compared to reports in cetaceans from other geographical locations.

1144 EFFECTS OF SUBLETHAL EXPOSURE TO DDT ON SURVIVAL AND REPRODUCTION OF THE MUMMICHOG (CYPRINID) *FUNDULUS HETEROCLITUS*

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The use of DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane) was banned in the United States in June of 1972. In less developed countries of the tropics (Africa, Central and South America and the Pacific Rim nations)however, DDT is still very intensively used for mosquito control and agricultural purposes. In this study, research was undertaken to examine the effects of DDT on a variety of life history stages of the mummichog, *Fundulus heteroclitus*. In preliminary experiments with adult mummichogs, the 96h LC50 of DDT was 20.3 ug/L. (14.40, 28.62). The No Observable Effect Concentration (NOEC) based on the acute exposure levels was 11.3ug/L. In subsequent experiments, adult mummichogs were induced to spawn in the laboratory by the administration of carp pituitary extract (CPE). The fertilized eggs were then exposed to a series of DDT concentrations (1.00, 2.00, 4.00, 8.00 and 16.00 ug/L) which bracketed the adult NOEC levels until hatching. All of these embryos successfully hatched. In a related experiment, fertilized eggs were exposed to DDT concentrations ranging from 4– 32ug/L. No effects on hatching success were observed, however all test concentrations

were lethal to the hatched larvae within 96 h of hatching. These results indicate that the NOEC determined for DDT in adult mummichog may not be relevant for more sensitive life history stages.

1145 ESTIMATION OF SENSORY IRRITATION POTENCY (I.E., RD50) IN MICE; ALTERNATIVES TO ANIMAL USE

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A multiple linear regression equation (MLRE) using physicochemical properties of nonreactive volatile organic chemicals (NRVOC) (e.g., alcohols, alkylbenzenes, ketones, esters) was used to estimate log RD50 (Alarie et al., TAP, 133; 92, 1995). The descriptors used in this equation are π_2^H, the chemical dipolarity/polarizability, $\Sigma\alpha_2^H$, the chemical overall or effective hydrogen-bond acidity and L (Oil), the chemical Ostwald partition coefficient on olive oil; all play a role in activation of the sensory irritant receptor. Using 56 NRVOC the equation is: log RD50 (ppm) = 6.72 -(0.79 × π_2^H) - (1.11 × $\Sigma\alpha_2^H$) - (0.85 × log L (Oil)), r^2 = 0.91. Using this equation as the "standard" mechanistic equation we have now obtained an empirical equation to predict RD50 on the basis of molecular weight (MW) alone, for NRVOC in the range of 32 to 170 MW. The equation is RD50 (ppm) = 5.40 - (0.021 × MW), r^2 = 0.61. We created a more versatile database by including chemicals with some reactivity such as saturated aldehydes and acetates, at the cost of a less reliable prediction: log RD50 (ppm) = (0.018 × MW), r^2 = 0.51. These equations are very useful to estimate log RD50 value for NRVOC. In particular, the calculation of MW is trivial in comparison to obtaining the descriptors in the MLRE. For a better estimate than with MW, L (Oil) can be easily measured. The equation for L (Oil) is: RD50 (ppm) = 6.01 - (0.81 × log L (Oil)), r^2 = 0.85. These are alternatives to animal use to estimate RD50.

1146 PREDICTING HUMAN CUMULATIVE IRRITATION SCORES USING AN IN VITRO HUMAN SKIN MODEL

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A global validation study has been developed which will compare human clinical 14-day cumulative irritation study results to in vitro results obtained using the Skin2® ZK1301 human skin model (Advanced Tissue Sciences, Inc., La Jolla, CA) and a protocol designed specifically for this study. Materials were chosen which represent the major surfactant classes and exhibit a wide range of irritation potential. The validation study consists of three phases: 1) training, 2) pre-validation, and 3) validation. Areas which are being addressed include test materials, human clinical irritation data, in vitro test system, in vitro protocol, in vitro results, and statistical approach. Initial data obtained in developing the in vitro protocol using 6 surfactants has shown favorable results. An 83.33% correlation has been obtained for the different classes of surfactant test materials to pilot human clinical 14-day cumulative irritation results. This in vitro prediction model is intended to serve as a pre-clinical screening test prior to clinical patch studies for novel ingredients and/or mistures.

1147 EFFECTS OF MPTP ON THE CHOLINERGIC SYSTEM OF HUMAN NEUROBLASTOMA SH-SY5Y CELLS

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Parkinsonism-inducing agent 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), recognized for its effects on the dopaminergic nervous system, was found to also affect the cholinergic system of SH-SY5Y cells. This human neuroblastoma cell line contains monoamine oxidase (MAO) for the metabolism of MPTP to 1-methyl-4-phenylpyridinium (MPP^+), which is considered the active neurotoxicant. Our studies showed that MPTP caused a dose-response decrease in the binding of [^{3}H]-methylscopolamine (^{3}H-NMS) at muscarinic receptors with IC_{50} 2.344 x 10^{-4} M, and also caused a decrease in [^{125}I]-a-bungarotoxin (^{125}I-Bgt) binding at nicotinic receptors with IC_{50} 1.950 x 10^{-5} M. The pretreatment with MAO inhibitors clorgyline and pargyline did not prevent the MPTP-induced decreases. Additionally, this experiment indicated that acetylcholine receptor antagonists atropine (10^{-6} M) and dtubocurarine (2 x 10^{-5} M) inhibited the MPTP-induced [^{3}H]-dopamine release from SH-SY5Y cells. We also found that MPTP inhibited acetylcholinesterase activity in a dose and time dependent manner (Ki 2.6455 x 10^{-3} M), which was not prevented by pretreatment with MAO inhibitors. These results suggest that MPTP itself could act on acetylcholine receptors as well as acetylcholinesterase, and that this may contribute to its neurotoxicity.

1148 HISTAMINE RELEASE FROM HUMAN LYMPHOCYTE PREPARATIONS BY THE VESICATING AGENTS, SULFUR MUSTARD AND LEWISITE

H L Meier. *USAMRICD, APG, MD Sponsor: S I Baskin*

Sulfur mustard (SM) and Lewisite (L) induce edema and urticaria that in humans results in bulla formation. An *in vitro* assay using human lymphocyte preparations containing basophils (lym) was employed to study the inflammatory component of vesicant injury. Lym exposed to either L or SM showed both a concentration- and time-dependent histamine release (HR) which was consistent with the reported development of lesions in humans. SM produced a time- and concentration-dependent biphasic HR response from the lym. SM (10^{-5} to 10^{-3} M) caused a low level (5–10%) HR between 4 and 12 hrs post-SM exposure and a concentration-dependent biphasic HR beginning at 16 hrs and increasing until 24 hrs. This second HR peak reached a max response (40–60% HR) between 3x10^{-5} and 10^{-4} M SM with decreasing levels of HR at higher and lower concentrations. SM concentrations which initiated max cytotoxicity (3x10^{-4} & 10^{-3} M) exhibited the lowest HR. L demonstrated a single phase time course of HR similar to that observed for cytotoxicity. L induced a concentration-dependent HR peak which was time dependent, but the percent of HR appeared to be constant over the concentration range. L at 10^{-3} M caused HR in the first 4 hrs which peaked by 8 hrs post-exposure. L at 10^{-5} M did not cause HR until 12 hrs post-exposure; this peaked at 24 hrs. L exposure levels in this range demonstrated peak time of HR proportional to concentration. The time course of SM-induced HR was independent of concentration, but the amount of HR was concentration dependent. The time course of L-induced HR was concentration dependent, but the percent HR was concentration independent. Although the time- and concentration-dependence of HR by both SM and L are very different, they appear to correlate with their respective time and concentration parameters for the induction of historically reported clinical signs which appear very similar.

1149 EFFECTS OF *IN VITRO* OZONE EXPOSURE ON EXPRESSION AND DISTRIBUTION OF INTEGRINS IN NASAL AND BRONCHIAL EPITHELIAL CELLS

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Integrins are a family of cell surface heterodimeric receptors involved in cell-substratum and cell-cell interactions. Changes in cellular expression and distribution of these molecules might disrupt interactions between lung epithelial cells resulting in increased permeability. The present study was designed to determine whether ozone exposure modulates the expression and distribution of adhesion molecules in cultured respiratory epithelial cells. Human nasal epithelial (HNE) cells, monkey bronchial (PBE) and nasal (PNE) cells were cultured at an air interface on collagen treated porous membranes until confluent. Cultures were then exposed to air, ozone, hydrogen peroxide, or cytochalasin D and prepared for immunocytochemistry using monoclonal antibodies to integrin subunit B_1, a_2B_1 and a_3B_1. Each cell layer was scanned using an attached cell analysis and sorting interactive laser cytometer to quantify total fluorescence and fluorescence at the apical and basal surfaces of the cells. A 2-fold increase in expression of the B_1 subunit was observed in PBE cells at 0 and 24 hrs after exposure to 0.5 ppm for 6 hrs compared to controls; however, apical and basal distribution was not altered. No change was also seen in the expression and distribution of a_3B_1 in HNE and PBE after ozone exposure. At 24 and 72 hrs following treatment, hydrogen peroxide caused a minimal increase in expression of a_2B_1 while cytochalasin D decreased significantly a_2B_1 expression. Neither agent had an effect on a_2B_1 distribution. These results indicate varying effects of ozone on certain integrins in respiratory epithelial cells. (Supported by NIH HL50580)

1150 THE BINDING OF [^{14}C]-1,3-DINITROBENZENE (DNB) BY A SPECIFIC MITOCHONDRIAL PROTEIN

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1,3-dinitrobenzene (DNB) is a testicular toxicant thought to cause damage after metabolic activation. The electrophilicity of reactive DNB metabolite(s) generated in the reduction of NO_2 to NH_2 suggests that binding by nucleophilic

cellular macromolecules might mediate DNB toxicity. One clearly radioactive protein was found in the testes after administration of ^{14}C-DNB *in vivo*, as well as after incubation of ^{14}C-DNB with whole seminiferous tubules and in Sertoli cell cultures. Subcellular fractionation localized this radiolabelled band to the mitochondrial fraction. ^{14}C-DNB-binding in liver slices was not limited to the mitochondrial fraction and radioactive bands were also associated with microsomal proteins. Isolated metabolically active mitochondria from either tissue also bound DNB in the presence of NADPH. When the DNB metabolite nitronitrosobenzene (NNB) was added to mitochondrial preparations the same protein was bound. However, this occurred only in the absence of the NADPH cofactor which supported the further reduction of NNB. Localization of this bound protein to the mitochondria is consistent with activation by redox processes. The labeled protein was 250,000 kDa by nondenaturing gel electrophoresis with subunit(s) of 52,000 by SDS PAGE. Work is continuing to identify this protein, and to determine whether the binding of DNB and the observed toxicity are related. Supported by NIH grant # ESO5701.

1151 GENDER DIFFERENCES AND TOXICITY

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Quantitative differences in response to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and other aromatic halogenated hydrocarbons, between species and between genders ranging from the time to respond to the effective dose. Quantitative distinct gender-specific actions of toxicants, particularly those that target reproductive tract organs, are recognized and exposure guidelines for reproductive toxicants have gender-specific exposure considerations. Gender-specific mechanistic differences in the action of toxicants on non-reproductive cells, however, have received little attention. We have found that TCDD induces mechanistic-specific-gender different effects in male and female guinea pigs in non-reproductive tract cells. For example, TCDD-induces a significant increase in cytosolic protein phosphorylation and in nuclear DNA binding activity of AP-1 in adipose tissue of male guinea pigs under all conditions including a *cell free* system. The opposite effect is seen in females. This gender difference is eliminated, however, when cytosolic protein from intact female animals are tested in a *cell free* system. As part of our investigations we also conducted a series of experiments using the intact and castrated adult male guinea pig treated with 10 ug estradiol/kg for five successive days followed by TCDD treatment for 24 h. The results of these studies support our hypothesis and underscore the importance of circulating steroids in the modulation of the effects of toxicants on non-reproductive tract cells particularly in castrated animals.

1153 MOLECULAR BASIS OF HMG CoA REDUCTASE INHIBITOR-INDUCED MYOTOXICITY

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In previous studies, inhibition of cholesterol synthesis by HMG CoA reductase inhibitors (HMGRI) was associated with myotoxicity in cultures of rat skeletal myotubes, and rhabdomyolysis in rats, rabbits and humans *in vivo*. *In vitro* myotoxicity, characterized by loss of myotubes and inhibition of protein synthesis, was directly related to HMGRI-induced depletion of mevalonate, farnesol and geranylgeraniol, since supplementation with these intermediate metabolites abrogated or markedly ameliorated the toxicity. Both farnesol and geranylgeraniol are required for the post-translational modification, or isoprenylation, of essential regulatory proteins in mammalian cells. The objective of the present study was to measure changes in protein isoprenylation in cultured rat skeletal muscle cells exposed for 24 hours to a range of concentrations of two HMGRI, pravastatin and lovastatin. Proteins were labeled with ^{3}H-farnesyl pyrophosphate (FPP), or ^{3}H-geranylgeranyl pyrophosphate (GGPP), separated by SDS-PAGE and quantitated by scintillation counting and densitometry. Fourteen protein bands were separated, with molecular weights ranging from 18 to 170 kDa. Labeling with FPP was very weak in the absence of HMGRI's, indicating dilution with endogenously synthesized farnesol. In contrast, GGPP labeling was equivalent in the presence or absence of HMGRI's, indicating a very low rate of endogenous geranylgeraniol synthesis. FPP-labeling of most protein bands increased at all concentrations of HMGRI tested, including concentrations that markedly inhibited protein synthesis, In contrast, FPP-labeling of three protein bands (26.9, 27.8 and 28.9 kDa) decreased with increasing concentrations of HMGRI, indicating that these three proteins require geranylgeranylation. The results of this study indicate that HMGRI-induced myotoxicity is most likely related to reduced post-translational modification of specific regulatory proteins by geranylgeraniol.

1154 EFFECTS OF GESTATIONAL AND LACTATIONAL EXPOSURE TO ORTHO-SUBSTITUTED PCBS, COPLANAR PCBS, OR TCDD ON LOCOMOTOR ACTIVITY IN RATS

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Many studies have shown that developmental exposure to polychlorinated biphenyls (PCBs) alters locomotor activity (LA), but most have focused on commercial PCB mixtures or coplanar PCB congeners. Studies of *ortho*-substituted PCB congeners are lacking. In a series of studies, we compared the effects of *ortho*-substituted PCBs, coplanar PCBs, a PCB mixture, and TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) on LA and rearing. Sprague-Dawley rats were dosed with PCB 28 (2,4,4'-trichlorobiphenyl), PCB 52 (2,2',5,5'-tetrachlorobiphenyl), PCB 118 (2,3',4,4',5-pentachlorobiphenyl), PCB 153 (2,2',4,4',5,5'-hexachlorobiphenyl), PCB 77 (3,3',4',4'-tetrachlorobiphenyl), PCB 126 (3,3',4,4',5-pentachlorobiphenyl), Aroclor 1242, TCDD or vehicle via gavage on gestation days 10-16. Litters were culled to eight on day 2 and weaned on day 21. On days 35, 65, and ~220, horizontal LA and rearing were assessed in an automated open field. Aroclor 1242 reduced LA, whereas several individual congeners including PCB 28, PCB 153, PCB 126, and TCDD increased LA. PCB 52 and PCB 118 were without effect. PCB 77 decreased LA in one study but had no effect in another. Similar effects on LA were observed in males and females. In contrast, effects on rearing were sex-related. PCB 126 and TCDD caused a marked increase in rearing in male rats, whereas various *ortho*-substituted PCBs appeared to decrease rearing in female rats. These findings demonstrate that developmental exposure to both *ortho*-substituted or coplanar PCBs can alter motor activity. The discrepancy between the effects of the Aroclor mixture (decreased LA) and the individual PCB congeners (increased LA) may be related to the fact that Aroclor 1242 contains over 70 individual congeners which could interact in complex ways to produce effects. Alternatively, decreases in LA caused by Aroclor could be due to PCBs that haven't yet been studies individually. Supported by Health Canada #3369 and 4028 to SLS and NIH #ESO1332 to REP.

1155 DEVELOPMENTAL DIOXIN EXPOSURE: DISRUPTION OF THYROID HORMONES AND OTOTOXICITY

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The developing auditory system has been shown to be sensitive to the thyrotoxic effects of PCB exposure (Goldey et al., Toxicol. Appl. Pharmacol., in press). We assessed the effects of developmental exposure to another known endocrine disruptor, dioxin, on auditory function. Primiparous rats (10/dose) received a single sc injection of dioxin, at 0 (DMSO vehicle), 0.3, 1, 3, or 10 ug/kg on GD19. We assessed offspring at a various age intervals for: plasma thyroid hormone, body weight, survival, motor activity, auditory startle amplitudes, auditory thresholds, and activity following an amphetamine challenge. Circulating T4 concentrations were reduced (up to 50%) in a dose-dependent fashion by PND 14 and 21. There was no effect of dioxin on T3 at any age. Postnatal pup mortality was 5–10% at the highest dose. No effects on motor activity were detected at any age, nor was there any differential effect of dioxin on amphetamine induced motor activity. Importantly, dioxin caused permanent hearing deficits (up to 20 dB threshold shift) at the lowest frequency tested (1 kHz), whereas auditory thresholds were not significantly affected at higher frequencies (4, 16, 32 or 40 kHz). These data support previous findings with PCBs and suggest a link between chemical-induced disruption of thyroid function and ontogeny of the cochlea.

1156 ESTROGENIC ACTIVITY OF 2,2',6,6'-TETRACHLOROBIPHENYL IN MCF-7 HUMAN BREAST CANCER CELLS

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There has recently been an increase in the public health concern regarding modulation of estrogenic function caused by exposure to polychlorinated

biphenyls (PCBs). Lower chlorinated, ortho substituted, non coplanar congeners are weakly estrogenic, and studies in animals and some *in vitro* assays suggest that meta or para hydroxyl substitution is obligatory. A systematic examination of the estrogenic potency of various PCB congeners has been undertaken to further characterize the structural requirements for estrogenic activity using the MCF-7 focus assay. This assay, based on estrogen-dependent, postconfluent cell proliferation in cultures of human MCF-7 breast cancer cells, results in development of discrete multicellular foci or nodules analogous to *in vivo* estrogen-mediated, proliferation-dependent, tissue restructuring. Dose-response studies indicate that 2, 2', 6, 6'-tetrachlorobiphenyl (TCB) displays estrogenic activity with an EC_{50} of 0.1 μM which is three orders of magnitude less potent than 17β-estradiol. This activity is suppressed by the estrogen-receptor blocking agent, LY 156758, suggesting that TCB is indeed acting through the estrogen receptor. Subsequent studies indicate that TCB is also estrogenic *in vivo* as shown by increased uterine wet weight (Seegal, Toxicologist 1995). Preliminary studies in MCF-7 cells failed to find any evidence of TCB hydroxylation. These results indicate that TCB is estrogenic in both a relevant novel *in vitro* human test system, and the traditional mouse uterotropic assay and that this activity may be intrinsic to the tetra ortho structure of TCB. (Funded by NIEHS ESO4913-06).

1157 ACUTE RESPONSES OF PREPUBERTAL RATS TO ENVIRONMENTAL PCBs WITH HIGH AND LOW DIOXIN EQUIVALENCIES

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A refined extract of landfill soil containing high levels of polychlorinated biphenyls (PCBs) was further refined by aluminacharcoal column chromatography to remove coplanar PCBs and high levels of chlorodibenzofurans. Both extracts were administered ip in corn oil to immature female rats on days 21 and 22 of age; rats were terminated on day 23 and organ weights, enzyme activities and total serum thyroxine (T4) were measured. The change in characteristics of the two mixtures was confirmed by comparing hepatic microsomal ethoxy-, pentoxy- and benzoxy-resorufin-O-dealkylase/dearylase activities [EROD, PROD, and BROD, respectively]. EROD activity, an estimate of Ah receptor agonists present, peaked at 151 mg/kg soil extract, declining at 226 mg/kg; EROD activity in rats treated with the charcoal-cleaned soil extract was not induced at low doses, but increased linearly from 78 to 248 mg/kg. Conversely, PROD and BROD activities were modestly induced at 6 to 78 mg/kg of either soil extract; however, at higher doses induction was 3-fold higher in rats receiving the charcoal-filtered extract. Liver weights increased and Phase 2 enzyme activities were induced in patterns similar to EROD. Liver cytosolic alanine amino-transferase (ALAT), a glucocorticoid regulated enzyme, was modestly induced in distinct dose-dependent patterns by the two soil extracts. At all doses, the uterotropic response was greater in rats receiving the charcoal-filtered extract, although not always significant; this relationship may have been due to the antiestrogenic actions of *Ah* receptor agonists removed by the charcoal. Total serum T4 declined similarly in rats receiving either extract to about 40% of control values at the highest doses. Short-term integrated bioassays such as this are useful in detecting multiple complex interactions and can be used to define the net effects of mixtures with changing compositions for improved risk assessment.

1158 MATERNAL EXPOSURE TO POLYCHLORINATED BIPHENYLS (PCB) ALTERS REGIONAL BRAIN GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) LEVELS IN RATS

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A previous study indicated that maternal exposure to Aroclor 1254 resulted in decreased levels of glial protein GFAP in the brainstem of the offspring. We conducted a reproductive study with Wistar rats in order to determine which class of PCB congeners in the commercial PCB mixture might have been responsible for the alterations in GFAP levels. Sperm-positive rats were exposed to a single oral does of PCB 126 (1.6, 4.9, 16,48 and 146 μg/kg), PCB 118 (0, 1.6, 4.9, 16, 48 and 146 mg/kg) and PCB 153 (18, 36, 72, 143 and 286 mg/kg) on day 1 of gestation. On postnatal day 90 male offspring were sacrificed and the brains were removed. Following brain dissection, brain regions were homogenized and assayed for protein concentration and GFAP content using a sandwich ELISA. All PCB 126 treatments resulted in a significant increase (40%) in GFAP levels (μg/mg protein) in the brainstem. Maternal exposure to PCB 153 resulted in significant increases (25%) in GFAP levels in the brainstem at 36, 72, and 143 mg/kg, but not at the highest dose, 286 mg/kg). Treatment with PCB 118 had no effect on GFAP levels in the brainstem, and none of the congeners tested altered GFAP levels in the cerebellum. We conclude that the effects of the individual congeners tested do not resemble that of Aroclor 1254.

1159 POLYCHLORINATED BIPHENYLS FOUND IN AROCLORS 1016, 1248, AND 1254 HAVE THE ABILITY TO REDUCE LONG-TERM POTENTIATION IN CA1 RAT HIPPOCAMPAL NEURONS

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The goal of this study was to examine the effects that some commonly found PCBs have on the functioning of hippocampal CA1 neurons. We monitored changes in long-term potentiation (LTP), a process associated with synaptic plasticity. Slices (400 μm) from Wistar rats were incubated in a 0.1% DMSO/Krebs containing 1 to 100 ppm of either Aroclor 1016, 1248, 1254 free-floating in a chamber with bubbled O_2/CO_2 for periods of 1 to 6 hrs. The average LTP amplitude decreased significantly for all exposures over 2 hr and 1 ppm. Eight of the 26 slices in the 1016 treated group and 8 of 18 slices in 1248 group had no LTP. Nineteen of 38 in the 1254 group had LTP of 10% or less (controls averaged 79%). Six of the 18 slices in the 1248 group had EPSP amplitudes that were initially less than controls and suffered a further decline during the 1 hr post-tetanus, resulting in a minus value for the LTP. This may reflect an effect on ion transport by the PCBs. Paired-pulse facilitation (PPF) and posttetanic potentiation (PTP) were significantly decreased in the 1248 treated cells. Supported by grant NIEHS 2P42ESO491306.

1160 DEVELOPMENTAL NEUROTOXICITY OF DEFINED PCB CONGENERS IN THE NEONATAL MOUSE - EFFECTS ON CHOLINERGIC AND BEHAVIOURAL VARIABLES

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Neonatal exposure to single PCB congeners 2,4,4'-trichlorobiphenyl (IUPAC 28), 2,2',5,5'-tetrachlorobiphenyl (IUPAC 52), 2,3',4,4', 5-pentachlorobiphenyl (IUPAC 118) and 2,3,3',4,4',5-hexachlorobiphenyl (IUPAC 156) indicated persistent neurotoxic effects from PCB 28 and PCB 52. The substances were given as one single dose (0.7 – 14 μmol/kg bw., roughly 0.2 – 5 mg/kg bw.) to 10-day-old male NMRI-mice. Aberrations in spontaneous motor behaviour were seen in adult mice (4 month old) exposed to PCB 28 and PCB 52. Neonatal exposure to PCB 52 also affected learning and memory functions in the adult animal (measured in radial arm maze and swim maze). In the animals showing deficit in memory and learning function, the cholinergic nicotinic receptors (NAChR) in the cerebral cortex were affected (assayed with (-)-[N-methyl-^{3}H]nicotine). This neonatal exposure to PCB 52 (14 μmol/kg bw.) also alters the spontaneous motor behaviour response to a cholinergic agent, nicotine, at adult age, but not to amphetamine, an agent known to affect the dopaminergic system. The effects on spontaneous motor behaviour and NAChR were observed when PCB 52 was administered on postnatal day 3 or 10, but not on postnatal day 19, indicating a limited period during neonatal life for the induction of permanent changes in adult brain function. Exposure to 2,3',4,4', 5-penta- and 2,3,3',4,4',5-hexachlorobiphenyl, mono-ortho congeners ('co-planar-like'), in the same dose-range did not cause any significant change in spontaneous motor behaviour or swim-maze behaviour.

1161 *ORTHO*-SUBSTITUTED POLYCHLORINATED BIPHENYLS ALTER MICROSOMAL Ca^{2+} TRANSPORT. EVIDENCE FOR A RECEPTOR-MEDIATED MECHANISM INVOLVING IMMUNOPHILIN FKPB12 AND RYANODINE-SENSITIVE Ca^{2+} CHANNELS IN EXCITABLE CELLS

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Studies have revealed that *ortho*-substituted polychlorinated biphenyls (PCBs) alter intracellular Ca^{2+} signaling in both primary neuronal cultures and PC12 cells, although the underlying molecular mechanism is unclear. Purified junctional SR membranes enriched in the skeletal isoform of ryanodine receptor (RyR1) and SERCA1 pump were used as a model to examine the mechanism by which PCB95 alters microsomal Ca^{2+} transport using radioligand-receptor

binding analysis and spectrophotometric measurements of active Ca^{2+} uptake and release with antipyrylazo III. Nonplanar PCB95 was found to enhance the binding of the conformation-sensitive channel ligand [^{3}H]ryanodine to RyR1, and to induce channel mediated Ca^{2+} release with EC_{50}s of 0.33 and 1.2μM, respectively. Immunophilin FKPB12, a 12kDa FK506 binding protein, is tightly associated with the RyR1 oligomer. Binding of FK506 to FKPB12 promotes dissociation of the immunophilin from the RyR1 channel complex. FK506 or rapamycin (20-100μM) were found to dose-dependently eliminate the ability of PCB95 to activate [^{3}H]ryanodine-binding sites. FK506 (50μM) also completely eliminated PCB95 induced Ca^{2+} release from actively loaded vesicles without interfering with responsiveness to caffeine, a FKBP12-independent channel agonist. These actions were found to be specific for PCBs with 2 or more *ortho*-chloro substituents. RyR isoforms and FKBPs are highly expressed in the mammalian CNS. Accordingly PCB95 was found to activate [^{3}H]ryanodine-binding sites in crude membrane preparations from cerebral cortex, hippocampus and cerebellum (EC_{50}s = 30μM) in the presence of physiological salt. These results show that *ortho*-substituted PCBs alter microsomal Ca^{2+} transport by a receptor-mediated mechanism involving the FKBP12/RyR1 complex and may be of significant toxicological importance.

1162 UTEROTROPIC AND NEUROCHEMICAL EFFECTS OF 2,2',6,6'-TETRACHLOROBIPHENYL IN THE PREPUBERTAL RAT

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Ortho-substituted polychlorinated biphenyls (*o*-PCBs) decrease dopamine (DA) concentrations *in-vitro* and in adult animals by inhibiting synthesis, presumably at the level of the rate limiting enzyme, tyrosine hydroxylase. Certain *o*-PCBs may also influence neurochemical function due to their estrogenic activity. Since 2,2',6,6'-tetrachlorobiphenyl (TCB) induces foci formation in MCF-7 human breast cells (an estrogenic response) (Gierthy, SOT '96), we have examined the effects of intraperitoneal exposure to TCB or 17β-estradiol (E_2) on uterine weight and neurochemical function in the prepubertal female rat. Rats, exposed on postnatal days (PND) 21 and 22 to either E_2 (20 μg/kg) or TCB (10, 20 or 30 mg/kg), were sacrificed on PND 23, and organ weights and brain biogenic amines were determined. E_2 and TCB significantly increased uterine wet weight and uterine/body weight ratios, but did not affect body, brain, liver or kidney weights. E_2 and TCB significantly elevated DA concentrations in the caudate nucleus while TCB significantly elevated DOPAC and HVA (DA metabolites) concentrations in the substantia nigra pars compacta. Given that: (i) TCB, in both *in-vivo* and *in-vitro* preparations is estrogenic and (ii) E_2 affects DA concentrations and metabolism in the basal ganglia, our data suggest that perinatal exposure to estrogenic PCBs such as TCB may play an important role in affecting the development of neurochemical indices of brain function. Supported in part by NIH grant ES04913.

1163 2,3,3',4,4'-PENTACHLOROBIPHENYL (PCB 105)-INDUCED HYPOTHYROXINEMIA IN RATS

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One of several mechanisms by which polychlorinated biphenyl (PCB) mixtures may act in Ah receptor-independent fashion is via conversion to hydroxylated metabolites that bind to thyroid hormone receptors and binding proteins. Our objective was to find a prototype PCB to investigate possible toxicological consequences of such interactions. PCB 105 was tested because 1) it is present in the environment, 2) it has relatively little Ah receptor agonist activity, and 3) its highly persistent 2,3,3',4',5-pentachlorobiphenyl-4-ol metabolite binds with high affinity to transthyretin. PCB 105 was synthesized via the Cadogan reaction and purified on silica gel columns, then tested using an *in utero* and lactational exposure protocol in order to expose animals when they are most vulnerable. Female Holtzman rats were dosed orally from Gestation Day 6 to Postnatal Day (PND) 21 with 0, 0.4, 1.0, or 2.5 mg PCB 105/kg/day. Dams appeared to be unaffected, and PCB 105 had little if any effect on survival, body weight, or thymus weight of pups. Plasma thyroxine concentrations in dams were not significantly affected on PND 21 but were reduced by >90% in male and female pups at this time (ED_{50} 0.5 mg PCB 105/kg/day). These results indicate that PCB 105 caused little dioxin-like toxicity even at the highest dose tested. In contrast, the Ah receptor agonist 3,3',4,4',5-pentachlorobiphenyl (PCB 126), when tested under the same protocol, caused a high incidence of perinatal mortality at >1.0 μg/kg/day. PCB 105 is therefore <1/2500th as potent as PCB 126 at causing overt toxicity in rats prior to weaning. The severe hypothyroxinemia caused by PCB 105, in combination with its minimal dioxin-like overt toxicity, demonstrate that PCB 105 is a suitable prototype for investigating Ah receptor-independent effects of PCBs on thyroid hormone status. The long term consequences of *in utero* and lactational PCB 105 exposure on reproductive and central nervous system development are now being assessed. (Supported by NIH ES06806)

1164 COMPARISON OF THE EFFECTS OF THE ORTHO-SUBSTITUTED 2,2',4,4' TCB AND THE "DIOXIN-LIKE" 3,3',4,4' TCB ON CATECHOLAMINE CONTENT AND RELEASE OF BOVINE CHROMAFFIN CELLS

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The effects of an ortho-congener (2,2',4,4' TCB) and a "dioxin-like" congener (3,3',4,4' TCB) on the catecholamine content and release of cultured bovine chromaffin cells were investigated *in vitro* by high performance liquid chromatography (HPLC). The K^+-stimulated catecholamine release was reduced by the 2,2',4,4' TCB, respectively 50 μM and 100 μM after a 5 days exposition and only by 100 μm after a 24 h exposition. This congener decreased the catecholamine content of the cells dose and time dependently, as revealed by Triton X-100 treatment. A comparison with the 3,3',4,4' TCB at the same conditions showed no reduction of catecholamine content. A slight increase of catecholamines was measured. The ratio between epinephrine (E) and norepinephrine (NE) in the disrupted chromaffin cells remained unaltered after the 3,3',4,4' TCB exposition but decreased after a 5 days exposition to the 2,2',4,4' TCB.

1165 DRUG DISCRIMINATION IN RATS FOLLOWING MATERNAL EXPOSURE TO A COPLANAR PCB OR PROPYLTHIOURACIL

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Previous experiments indicated that after treatment with the dopamine D_2 receptor blocker haloperidol the resulting catalepsy was more pronounced in rats with maternal exposure to the coplanar PCB 3,3',4,4'-tetrachlorobiphenyl (3,4-TCB) than in rats exposed to the ortho-chlorinated congener 2,4-TCB and controls. In the present experiment drug discrimination in a standard two-lever task was examined in rats maternally exposed to 3,4-TCB using apomorphine at a dose reported to act on D_2 receptors. Since impairment of thyroid function is suggested to mediate neurobehavioural effects of PCBs a group with perinatal exposure to propylthiouracil (PTU) was included as a positive control. PTU-treated rats needed more daily sessions than controls to reach the criterion for successful discrimination between apomorphine and saline. Differences between controls and groups exposed to 3,4-TCB or PTU were detected in a blocking test using the serotonin 5-HT_{1A} agonist buspirone which is also a partial antagonist to the D_2 receptor. This outcome suggests an effect of 3,4-TCB on the interaction between dopaminergic and serotonergic processes.

1166 INCREASED [^{3}H]PHORBOL ESTER BINDING AND INHIBITION OF $^{45}Ca^{2+}$-SEQUESTRATION BY HYDROXYLATED PCB CONGENERS: STRUCTRURE-ACTIVITY RELATIONSHIPS

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Our previous structure-activity relationship studies indicated that the effects of polychlorinated biphenyls (PCBs) on neuronal Ca^{2+}-homeostasis and protein kinase C (PKC) translocation were associated with the extent of coplanarity. Chlorine substitutions at *ortho*- position on the biphenyl, which induces non-coplanarity, are characteristic of the most active congeners *in vitro*. In the present study, we investigated the effects of selected hydroxylated PCBs, which are major PCB metabolites identified in mammals, on the same measures where PCBs had differential effects based on chlorine substitution patterns. These measures include PKC translocation as determined by [$_3$H]phorbol ester ([^{3}H]PDBu) binding in cerebellar granule cells, and Ca^{2+}-seques-

tration as determined by $^{45}Ca^{2+}$-uptake by microsomes isolated from adult rat cerebellum. All the hydroxy PCBs with *ortho*-chlorine substitutions increased [^{3}H]PDBu binding in a concentration-dependent manner and the order of potency as determined by E50 (concentration that increases control activity by 50%) is 2',4',6'-trichloro-4-biphenylol (32 ± 4 μM) > 2',5'-dichloro-4-biphenylol (70 ± 9 μM), 2,2',4,5,5'-pentachloro-4-biphenylol (80 ± 7 μM) and 2,2',5'-trichloro-4-biphenylol (93 ± 14 μM). All the selected hydroxy PCBs inhibited microsomal $^{45}Ca^{2+}$-uptake to a different extent. Among the hydroxy PCBs selected, 2',4',6'-trichloro-4-biphenylol is the most active in increasing [^{3}H]-PDBu binding as well as inhibiting microsomal $^{45}Ca^{2+}$-uptake. 3,5-Dichloro-4-biphenylol and 3,4',5-trichloro-4-biphenylol did not increase [^{3}H]PDBu binding, but inhibited microsomal $^{45}Ca^{2+}$-uptake. This effect was not related to ionization of these two hydroxy PCBs. Compared to parent PCBs, the hydroxylated PCBs seemed to be more active *in vitro*. These studies indicate the possible involvement of PCB metabolites in the neurotoxic response of PCBs.

1167 APOPTOSIS INDUCED BY GLUCOCORTICOIDS ON INTERLEUKIN-2 DEPENDENT LYMPHOCYTES INVOLVES A TRANSCRIPTIONAL MECHANISM

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Glucocorticoids (GC) modulate the immune response by inhibiting cytokine gene expression and by inducing apoptosis on lymphoid cells. Some discrepancies recently appeared concerning whereas transcription is an absolute requirement for GC-induced apoptosis. In some models, truncated glucocorticoid receptor (GR) lacking the N-terminal domain, needed for GC-dependent transcription, are still able to induce apoptosis probably through a transrepressive effect. In this work, we evaluated the respective involvement of transcription and transrepression in GC-induced apoptosis on CTLL-2 lymphoid cells. Actinomycin D and cycloheximide inhibited dexamethasone (DEX)-induced apoptosis as assessed by DNA gel electrophoresis suggesting that transcription and translation are necessary. We then compared the respective effects of DEX and RU 38486, which binds to GR but does not induce transcription, to provoke apoptosis. Using flow cytometry and propidium iodide, we demonstrated that cells treated with DEX but not with RU 38486 showed features of apoptosis Furthermore, both molecules were able to inhibit AP-1 DNA binding activity in a gel retardation assay showing that DEX and RU38486 possessed an intact transrepressive function. This result was confirmed using a 5X-TRE plasmid transiently transfected in CTLL-2 cells to assess AP-1 transcriptional activity. In conclusion, we demonstrated that transcription is an absolute requirement in the mechanism of apoptosis induced by GC in IL-2 dependent lymphoid cells whereas transrepression is not involved.

1168 AH RECEPTOR EXPRESSION IN BONE MARROW STROMAL CELLS INFLUENCES DMBA-INDUCED PRO-B CELL APOPTOSIS

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PAH are environmental contaminants which are immunosuppressive in whole animal studies, possibly through aryl hydrocarbon receptor (AhR) interaction. However, the exact mechanism of PAH immunotoxicity has not been completely defined. The present study was designed to determine the mechanisms by which PAH influence apoptosis of a stromal cell-dependent proB cell line (BU-11). Addition of the PAH, DMBA, at 10^{-9} - 10^{-6} M to cultures of BU-11 cells on a cloned bone marrow-derived stromal cell (BMS2) results in dose-dependent apoptosis in BU-11 cells at 24 hours. α-Napthoflavone (ANF), an AhR antagonist and cytochrome P-450 inhibitor, blocked DMBA-induced apoptosis implicating a role for the AhR and/or P450 in apoptosis. Western blots, *in situ* hybridization, and RT-PCR indicate that BMS2 but not BU-11 cells express high levels of AhR protein and mRNA, suggesting that AhR-mediated apoptosis in BU-11 cells may be effected indirectly through AhR-high BMS2 stromal cells. To test this hypothesis, the ability of DMBA to induce apoptosis in BU-11 cells maintained in IL-7 and without BMS2 cells was assessed. BU-11 cells maintained in IL-7 were resistant to DMBA-induced apoptosis. However, addition of BMS2 to BU-11 cells in the presence of IL-7 reconstituted DMBA sensitivity. These results suggest that AhRhigh stromal cells are required for DMBA-induced apoptosis in AhRlow or AhR- proB cells, which are intrinsically resistant to DMBA, and that apoptosis is not due to loss of stromal cell function. Further studies include determination of stromal cell factors which induce proB cell death and the elucidation of PAH-induced, proB cell apoptosis pathways. Funded by Grant # RO1-ESO6086.

1169 INHIBITION OF POLY(ADP-RIBOSE) POLYMERASE FACILITATES ACTIVE CELL DEATH IN OXIDANT-STRESSED ENDOTHELIAL CELLS

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Endothelial cells (EC) are subject to oxidative-induced cell injury and cell death during a variety of toxicities and pathologies. In EC, activation of poly(ADP-ribose) polymerase (PARP) is involved in mediating oxidant-induced cell death, putatively by causing a depletion of the PARP substrate, NAD^+ and cellular energy stores. In the current study, EC were exposed to various concentrations of H_2O_2 and viability (loss of membrane integrity) was continuously monitored using YO-PRO-1 fluorescent dye exclusion. The magnitude and onset of cell death following exposure to H_2O_2 were concentration dependent, with the earliest onset of cell death occurring 2–3 hr after H_2O_2 addition. When EC were treated with the PARP inhibitor, 1,5-dihydroxyisoquinoline, the time-course of cell death was delayed, with the onset of EC lysis beginning 4–5 hours following the initial oxidative damage. Addition of the protein synthesis inhibitor, cycloheximide to PARP-inhibited cells further delayed the onset of lysis (7–8 hr) and diminished the extent of cell death. Similarly, when PARP-inhibited cells were exposed to the endonuclease inhibitor, aurintricarboxylic acid, the extent of cell death was also diminished. Two biochemically and morphologically distinct modes of cell death, necrosis and apoptosis, are recognized as potential pathways of cell death following a toxic insult. One hallmark of apoptosis is that it is an active, energy requiring process involving gene transcription and translation. ***We hypothesize that the oxidant-induced EC death, which occurs following inhibition of PARP, is mediated by an active, energy-requiring process suggestive of apoptosis***. Therefore, PARP inhibition following oxidative DNA-damage maintains cellular energy stores and modulates whether a cell undergoes a necrotic or an apoptotic mode of cell death.

1170 DMBA-INDUCED APOPTOSIS OF B LYMPHOCYTES FROM MURINE BONE MARROW CULTURES

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The effects of dimethylbenzanthracene (DMBA) on the immune system and specifically the hematopoietic tissues have yet to be fully elucidated, although apoptosis has been implicated as a mechanism for depletion of lymphocytes by some polycyclic aromatic hydrocarbons. This depletion may cause a loss of immunocompetence detrimental to both animals and humans. Described here is a set of studies aimed to identify the response of primary murine bone marrow cultures to DMBA treatment and the components involved. The system involves C57B1/6 mouse-derived bone marrow cultures containing both an adherent stromal layer and a stromal cell adherent preB cell component. In a dose and time dependent manner, DMBA causes the preB cells from these cultures to apoptose, as determined by flow cytometry and DNA gel electrophoresis. At the optimal dose (10^{-4}M) and time (48 hours), the apoptosis after DMBA treatment was greater than 10-fold that of vehicle-treated cultures (58.8% ± 6.4 and 4.6% ± 1.2, respectively). This effect could be completely inhibited by α-napthoflavone, a competitive inhibitor of the aromatic hydrocarbon receptor (AhR) and the P450 enzymes, CYP1A1 and CYP1A2. In addition, to assess the role, if any, of the AhR in the induction of apoptosis, the level of AhR mRNA was determined by RT-PCR and in situ hybridization in each of the culture components. The amount of AhR in the stromal layer was found to be significantly greater than in the B lymphocytes. These results suggest that DMBA is signaling B cells to die either via the AhR in the stromal layer or via AhR-independent constituitive P450 enzyme activity in preB cells. Supported by RO1-ES06086

1171 PAH-INDUCED APOPTOSIS IN BONE MARROW B CELLS REQUIRES SUPPORTING STROMAL CELLS CONTAINING THE AH RECEPTOR

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Accumulated experimental data indicate that the Ah receptor mediates immu-

notoxicity of several environmental pollutants including PAH. Previously, we described one manifestation of PAH immunotoxicity in the induction of apoptosis in bone marrow preB cells supported by stromal cells. To examine the role of the AhR in immuntoxicity including apoptosis, AhR expression in cells that participate in immune response were assayed by RT-PCR. Furthermore, the role of the AhR in immature B cell apoptosis was determined using an immature B-cell line (BU-11) that was exposed to DMBA or B[a]P while supported by different feeder cell lines varying in AhR expression and/or function. Primary bone marrow derived preB cells, the immature bone marrow B-cell line BU-11, a mature B cell line (WEHI-231), and several T cell hybridomas were shown to express little or no AhR mRNA. In contrast, bone marrow derived stromal cells, monocytes and a monocyte line express relatively high AhR mRNA levels, suggesting that at least some immunotoxicity may occur secondary to PAH effects on accessory populations. Consistant with this hypothesis, BU-11 were shown to undergo apoptosis in response to PAH when maintained on bone marrow stromal cells (BMS2) or hepatocytes (Hepa-1c1) expressing AhR-mRNA, but not when grown in feeder cell conditioned supernatant or on AhR$^-$ feeder lines such as STO fibroblasts. We conclude that supporting stromal/feeder cells provide an active and required component for PAH-induced apoptosis. These data support the hypothesis that PAH immunotoxicity is not necessarily directed at lymphocytes *per se* but at the accessory cells on which lymphocytes depend for growth and/or function.

1172 4-HYDROXYNONENAL INDUCED CELL DEATH IN MURINE LUNG CELLS

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Oxidative stress is known to cause peroxidation of polyunsaturated fatty acids. 4-Hydroxynonenal (HNE) is a diffusible product of lipid peroxidation, and has been shown to be toxic to different cell types. In this study, the effects of HNE on lung cells from two murine strains (C3H/HeJ and C57BL/6J) were examined. HNE induced the formation of cellular HNE-protein adducts in cells from both strains of mice in a dose-dependent manner, and the amount of HNE protein adducts formed in cells from both strains were very similar. In the HNE dose range from 1 to 100 μM, cells from both strains had very little necrosis as shown by trypan blue staining. However, cells from both C3H/HeJ and C57BL/6J mice had extensive apoptosis at 100 μM HNE and little or no apoptosis at 25 μM HNE. Interestingly, the cells from C57BL/6J mice had significant apoptosis at 50 μM HNE while cells from C3H/HeJ mice had no significant apoptosis at this dose. At low dose of HNE, there was induction of heme oxygenase I. The data indicated that HNE induces apoptosis in murine macrophages, and cells from different strains of mice have different sensitivities to the HNE-induced apoptosis. The cause of the difference in susceptibility is not known, but it is possible that the different stress response may be in part responsible for it. Our observation that the product of lipid peroxidation causes apoptosis suggested that it might be a mediator for oxidative stress-induced apoptosis.

1173 APOPTOSIS AS A MECHANISM OF TRIMETHYLTIN INDUCED CELL DEATH IN A HUMAN NEUROBLASTOMA CELL LINE

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Trimethyltin (TMT) is highly neurotoxic after *in vivo* application, causing neurobehavioural changes and triggering neuronal degeneration in specific areas of the CNS. The interaction of this compound with SH-SY 5Y, a human neuroblastoma cell line, has been investigated. SH-SY5Y were exposed to TMT (0.5–10 μM) for 6, 24 and 48 hrs. TMT 5μM induced cell death and nuclear changes typical of the apoptotic event, evident 24h after the exposure. Removal of intracellular Ca^{2+} by BAPTA, prevented both cell death and apoptosis induced by TMT in SH-SY5Y cells.

TMT did not affect human leukemia cells (HL 60) viability, differently from triethyltin (TET), another neurotoxic organotin compound. Nevertheless, TMT induces cell death in other cell line of neural derivation: PC12 cells (EC50 = 10μM) and cerebellar granular cells (EC50 < 5μM).

These results indicate that TMT induces apoptosis in a neuroblastoma cell line activating a Ca^{2+} dependent pathway and that its effect is higly specific for neural cells. It is thus conceivable that this mechanism of cell death could account for the degenerative process observed *in vivo* after organotin exposure.

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1174 OXIDATIVE AND REDUCTIVE STRESS TRIGGER APOPTOSIS IN RENAL EPITHELIAL CELLS

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Oxidative and reductive stress have been shown to induce irreversible cell injury and cell death by necrosis. We now show that oxidative stress secondary to iodoacetamide (IDAM) treatment and reductive stress caused by dithiothreitol (DTT) induce apoptosis in the renal epithelial cell line LLC-PK1. IDAM causes rapid depletion of cellular non-protein thiols (NPSH), lipid peroxidation (LPO) and lactate dehydrogenase (LDH) release indicative of necrosis. The antioxidant *N,N*-diphenyl-*p*-phenylenediamine (DPPD) prevents LPO and LDH-release but not the depletion of NPSH (*Arch. Biochem. Biophys. 284: 422–430, 1991*). IDAM did not cause apoptosis in the absence of DPPD, but with DPPD present, IDAM induced apoptosis as determined biochemically (cleavage of DNA into ~200bp fragments) and morphologically (chromatin condensation and nuclear fragmentation). Brief treatment (15 min) with DTT (10 mM) after IDAM exposure prevented IDAM-induced apoptosis and reversed the depletion of protein thiols suggesting that oxidation of cellular (non)protein thiols initiates apoptosis. However, prolonger exposure to DTT (10 mM) induced reductive stress and apoptosis. Interestingly mild treatments which activate *gadd153* and *grp78*, two reductive stress-responsive genes, protected against IDAM-induced apoptosis. In conclusion, oxidative and reductive stress can both serve as signals for apoptosis, but the components of the reductive stress response can prevent oxidative-stress induced apoptosis.

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1175 ACTIVATION OF C-MYC AND REPRESSION OF BETA-ACTIN BY ADRIAMYCIN IN THE HEART OF FVB MICE

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Cardiotoxicity of Adriamycin (ADR) is a major limiting factor for clinical application of this important anticancer drug. The cellular and molecular mechanisms for the ADR-induced heart injury are unknown. The present study was undertaken to investigate cellular events and molecular alterations in the heart of ADR-treated FVB mice. Animals were treated with ADR (20 mg/kg) by a single ip injection. Twenty four hours or four days after the treatment cardiac lipid peroxidation and expression of genes that have been shown to be altered in apoptosis, such as c-myc and β-actin, were determined. Northern blot analysis showed that c-myc was activated at 4 days while no detectable change was observed at 24 hours. Beta-actin gene was repressed at both 24 hours and 4 days. Lipid peroxide levels were increased at 4 days. Different from transient and early response to mitogens, the lag expression of c-myc has been shown to be involved in apoptosis in several biological systems. Repression of β-actin was found in the induction of apoptosis in some cultured cells and depression of actin is required for apoptosis of Lepidoptera intersegmental muscle. Lipid peroxidation is also commonly involved in apoptosis. Taking together, the findings suggest that ADR may induce apoptosis in the heart of mice, which may be a major event of ADR cardiotoxicity. Supported in part by NIH Grants CA68125 and CA63752.

1176 ACTIVATION OF TRANSCRIPTION FACTOR NF-kB IS INVOLVED IN APOPTOSIS INDUCED BY TRIPHENYLTIN IN HL-60 CELLS

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The pesticide triphenyltin chloride (TPT) causes apoptosis in different cell types. The onset of this phenomenon is generally thought to require the induction of a novel genetic program. In this study we evaluated the link existing between activation of the transcription factor NF-*k*B and eventually apoptosis in HL-60 cells exposed to TPT. Dose-dependent DNA fragmentation of HL-60 cells after 6h of tratment with 2.5 or 5 μM TPT was observed. Apoptosis was confirmed by detection of apoptotic bodies 3 h after TPT treatment. Activation (15-30 min) of NF-*k*B induced by 5 μM TPT preceded both the appearance of apoptotic bodies and DNA fragmentation. NF-*k*B activation by TPT was inhibited by pretreatment of HL-60 cells with 100 μM of pyrrolidine dithiocarbamate (PDTC), a metal chelator that inhibits the generation of reactive oxygen intermediates (ROI). In the meantime, 100

μM PDTC protected the cells from DNA fragmentation induced by TPT. These findings suggest that TPT-induced apoptosis in HL-60 cells might be mediated by NF-*k*B activation, through the generation of reactive oxygen intermediates. Acknowledgment: this work was partially supported by an EC Grant contract EV5V-CT94-0508 and by a MURST project 40%

1177 OZONE-INDUCED APOPTOSIS IN HUMAN LUNG CELLS

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Ozone is a photochemically-generated pollutant that can produce acute pulmonary inflammation, and may contribute to the development or exacerbation of chronic lung diseases. However, the mechanisms of injury are still uncertain. In this study, human subjects were exposed for 1 hr to 0.4 ppm ozone (each subject also served as their own control with air exposure) with moderate exercise then underwent airway lavage (AL) and bronchoalveolar lavage (BAL) 6 hr post exposure. Ozone exposure induced apoptosis (morphology and Cell Death ELISA) in AL macrophages, but not in BAL macrophages. However, in BAL macrophages following ozone exposure, there was induction of HSP-72, hemeoxygenase-1, and ferritin, as well as, the presence of 4-hydroxynonenal (HNE) protein adducts (all determined by Western analysis). Low doses of HNE (5-25 uM) induced stress proteins, while higher doses (50-100 uM) were required to induce apoptosis in BAL cells *in vitro*. Therefore, we propose that ozone can produce variable responses in lung macrophages depending on location and expected ozone delivery to that location, and that these effects are consistent with HNE serving as a potential mediator of these effects.

1178 SILICA-INDUCED APOPTOSIS MEDIATED VIA SCAVENGER RECEPTORS IN ALVEOLAR MACROPHAGES

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The pathophysiological effects of silica depend on the particile size and on the physiochemical properties of the particulate surface. The negative charges on silica may have an increased and unique potential to adsorb and interact with cellular components. Macrophage scavenger receptors (SR) are trimeric integral membrane proteins exhibiting unique ligand-binding characteristics. The SR is speculated to play an important role in the uptake of charged particulates (negatively charged silica) by alveolar macrophages. In this study we examined the effects of fibrogenic particulates such as silica on alveolar macrophages obtained by bronchoalveolar lavage from human subjects. Cells were treated with crystalline silica (highly fibrogenic), amorphous silica (poorly fibrogenic) and titanium dioxide (non-fibrogenic) *in vitro* for 6 and 24 hrs. Treatment with crystalline silica resulted in significant alveolar macrophage apoptosis, whereas, treatment with amorphous silica and titanium dioxide did not result in apoptosis. We further investigated the role of scavenger receptors in silica-induced apoptosis using a SR ligand binding inhibitor, Poly I. Pretreatment with Poly I resulted in complete inhibition of silica-induced apoptosis. These results suggest that fibrogenic particulates such as silica cause apoptosis of alveolar macrophages via interaction with the SR and that this apoptotic potential of fibrogenic particulates may be a critical factor in initiating an inflammatory response resulting in fibrosis.

1179 DIFFERENTIAL ACTIVATION OF THE TRANSCRIPTION FACTOR c-MYC IN RESPONSE TO CHEMICAL STRESS

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The *c-myc* gene is an "immediate early" gene which is activated rapidly by signals for growth or apoptosis. The c-MYC protein is a transcription factor which cooperates with MAX and MAD to regulate transcription of genes containing CACGTG (E box) elements. S-(1,2-dichlorovinyl)-L-cysteine (DCVC) activates *c-myc* expression in the renal epithelial cell line LLC-PK1, therefore, we used DCVC to investigate *c-myc* and MYC target genes regulation by chemical stress. *c-Myc*, but not *max* or *mad*, mRNA and c-MYC protein were induced by DCVC. Ornithine decarboxylase (ODC) is a MYC target gene and DCVC induced both ODC activity and *odc* mRNA expression. We also prepared LLC-PK1 cells carrying a murine *odc* promoter-luciferase constructs containing either two wild type (pODC.WT) or mutant (pODC.MT) E-boxes. DCVC treatment induced luciferase activity 5–6 fold in PK1pODC.WT cells, but only 2–3 fold in PK1pODC.MT cells. LDH release and activation of pODC.WT and pODC.MT were both blocked by amino(oxyacetic) acid, a β-lyase inhibitor, but antioxidants blocked only cell death. Although S-(1,1,2,2-tetra-fluoroethyl)-L-cysteine, iodoacetamide and t-butyl-hydroperoxide induced *c-myc* mRNA, only DCVC induced MYC protein and pODC.WT luciferase activity markedly. Thus, activation of c-MYC and its target genes by chemical stress requires specific chemical and biological properties. The biological consequences of c-MYC activation by toxicants are under investigation.

1180 THE UTILITY OF MAMMALIAN GENE CONVERSION FOR THE DETECTION OF GENOTOXICITY

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Studies in yeast and fruit flies have demonstrated that DNA damage can induce gene conversion. In an attempt to exploit this we developed transgenic mice to detect germline gene conversions. The system is based on two mutually defective reporter (*lacZ*) genes under the control of a spermatogenesis-specific promoter. An intrachromosomal gene conversion event must occur for the generation of functional *lacZ* activity in spermatids. In OPP2 transgenic mice (which carry an array of approximately 10 copies of the transgene) injected with a single dose of 10 mg/kg of cisplatin, we observed a significant increase in the number of blue spermatids at day 14 post-injection. In the C57BL/6J genetic background, untreated transgenics demonstrate a level of about 0.001% *lacZ*-positive cells. Cisplatin treatment caused nearly 20% of the spermatids to stain blue. This increase, however, returned to baseline by day 28 post-injection, indicating that cisplatin does not affect the germinal stem cells. To provide molecular corroboration that the positive cells did, in fact, undergo cisplatin-induced gene conversion, *lacZ* positive (blue) and *lacZ* negative (white) sperm cells were isolated by micromanipulation and pooled for PCR analysis. The results of which confirmed that the blue cells indeed contained converted recipient molecules, but only about 10% of them appeared to have the converted template. Hence, the *lacZ* staining appears to have overestimated the degree of gene conversion by 10 fold. Nevertheless, this indicates that the true conversion rate was about 2% of spermatids, which is a 2000 fold induction over the 0.001% basal rate. Moreover, in parallel experiments utilizing COR3 homozygotes (which have only a single copy of the transgene) we observed a concentration dependent (5–10mg/kg) increased in the number of blue sperm cells at day 14 in cisplatin exposed mice. In contrast, COR3 mice injected with 2,3,7,8-TCDD (25 μg/kg) showed no increase in the number of blue cells.

1181 INHIBITION OF HUMAN TOPOISOMERASE II *IN VITRO* BY DIPHENOQUINONE, A PEROXIDASE-ACTIVATED METABOLITE OF BENZENE

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Benzene is a known human leukemogen whose mechanism of action remains a topic of intensive research. Recently, several phenolic metabolites of benzene have been shown to inhibit the enzyme topoisomerase II *in vitro* in the presence of a peroxidase activation system. Activated 4,4'-biphenol, a peroxidase metabolite of phenol exhibited the most potent inhibitory activity. To determine whether diphenoquinone (DPQ), an oxidative metabolite of 4,4'-biphenol, was responsible for inhibition, DPQ was synthesized and the structure verified by mass spectrometry. DPQ was then tested for direct inhibitory effects on human topoisomerase II utilizing a decatenation assay. Inhibition was seen, but the inhibitory concentration was found to be heavily dependent on assay conditions. Based on these results we retested a series of benzene metabolites. The phenolic metabolites, 4,4'-biphenol, 2,2'-biphenol, phenol, hydroquinone, catechol and 1,2,4-benzenetriol required peroxidase bioactivation, while the reactive benzene metabolites 1,4-benzoquinone and *t, t*-muconaldehyde were directly inhibitory using the modified assay conditions. All the metabolites were found to inhibit topoisomerase II in the low micromolar (~10 μM) concentration range. This research was conducted in part under contract to the Health Effects Institute (HEI).

1182 GENOTOXICITY STUDIES OF ALACHLOR

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The weight of evidence from well-validated assays conducted to assess genotoxicity of alachlor indicates that alachlor does not have general genotoxic potential. Alachlor and urine and bile samples from alachlor-treated rats were not mutagenic in Ames assays. Alachlor was negative when tested up to cytotoxic levels in a CHO/HGPRT mammalian cell gene mutation assay and was also negative in *in vivo* rat bone marrow cytogenetic and micronucleus assays conducted at dose levels up to 1000 mg/kg p.o. and 600 mg/kg i.p, respectively. Alachlor was negative in *in vivo/in vitro* rat UDS assays at dose levels below 1000 mg/kg. Variable responses were observed at an oral dose of 1000 mg/kg, a dose level that causes severe hepatotoxicity. Further studies have focussed on nasal tissue, a target site for induction of tumors in the rat. Nasal turbinate S9 metabolic activation systems from rats, mice and monkeys did not activate alachlor, an alachlor secondary sulfide metabolite or diethyl aniline to mutagens detectable in the Ames assay. These studies support the conclusion that alachlor's rat-specific oncogenicity does not result from a DNA reactive mechanism or genotoxicity but rather from alachlor's effects on non-genotoxic processes.

1183 THE STUDY OF ONCOGENE ACTIVATION AND DNA ADDUCTS FORMED BY THE EXTRACTS OF AIR SUSPENDED PARTICLES

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In this research, The samples were collected with high- - volume samplers on glass fiber filters, and extracted with organic solvent. Nontransformed SHE fibroblasts and lung cells were isolated from 12-day -old embryos. After treatment with the extract of TSP, the cells were cultured until reaching confluence, and then extracted DNA from it. DNA adduct was assayed with[32] P- postlabeling technque assay. RFLP was analysis with EcoR 1/Hind III treatment. Ras sequences were amplified by polymerase chain reacton (PCR), then the samples were analysed on 8% acrylaminde/urea gels. Southern blotting and hybridization experments were performed. The results showed that DNA adduct were formed and point mutations were also found in the cells. It may indicate that DNA adduct is involved in the ras gene activatiion in cells.

1184 ANALYSIS OF DNA STRAND BREAKS INDUCED IN VIVO AND *IN VITRO* BY TRIHALOMETHANES (THMs)

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The trihalomethanes are found in finished drinking water as by-products (DBPs) of the disinfection process. The exposure of large populations over long time periods to low concentrations of THMs has raised concern about the safety of chlorinated drinking water. Chloroform (CHCl3) has been uniformly negative in genetic toxicity assays; the data for the genotoxicity of the brominated THMs, bromoform (CHBr3), chloro-dibromomethane (CHBr2Cl) and bromodichloromethane (CHBrCl2) have been conflicting. We studied the ability of the THMs to induce DNA strand breaks (SB) using the DNA alkaline unwinding assay [1] after administration to rats and mice by gavage (aqueous) and in drinking water and [2] in rat hepatocytes and CCRF-CEM cells exposed *in vitro*. Exposing CCRF-CEM cells to 1, 5, and 10 mM THMs for 2 hr produced DNA SB. The order of activity was CHBr>CHBr2Cl>CHBrCl2; CHCl3 was inactive. After a 22 hr recovery period, cells exposed to ≥5 mM CHBr3 or 10 mM CHBr2Cl did not recover. Treating rat hepatocytes in primary culture with 1 – 10 mM CHBr3 and CHBr2Cl for 4 hr failed to induce DNA SB without concomitant cell toxicity. CHBrCl2 and CHCl3 produced no toxicity or SB. Brominated THMs (0.3 and 0.6 mmol/kg; 4 hrs) administered by aqueous gavage to male B6C3F1 mice and F344 rats failed to increase the number SB above background in the DNA of liver, kidney, and duodenum. CHBr3 (1.25 g/l) and CHBr2Cl (1 g/l) in the drinking water of mice for 2 weeks increased DNA SB in the duodenum. CHBrCl2 failed to increase DNA SB in any of the three tissues from the mouse (0.75 g/) after 2 weeks of exposure or the rat (0.6, 1.2, and 2.4 g/l) following a 5 week exposure period. These data demonstrate that the brominated THMs administered in an aqueous vehicle possess a specific type of genotoxicity (DNA SB) in cells with low metabolic capability (CCRF-CEM, duodenal epithelial cells), but not in cells with an active P450 enzyme system (hepatocyte, kidney tubule). However, metabolism by gut microbes cannot be ruled out in the case of CHBr3 and CHBr2Cl genotoxicity in the duodenal cells. *This abstract does not necessarily reflect EPA policy.*

1185 CELLULAR RESPONSES IN HUMAN KERATINOCYTE CULTURES AFTER EXPOSURE TO LOW DOSES OF MUSTARD COMPOUNDS

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The dose-dependent appearance of single-strand breaks (SSB) in the DNA of cultured rat keratinocytes immediately after exposure to 0.1–15 μM Bis-(2-chloroethyl) sulfide (BCES) has been reported (Toxicol. Appl. Pharmacol., 111, 342-351, 1991). These SSB were repaired by 22 hrs. post-exposure to as much as 5 μM BCES. However, [^{3}H]deoxyguanosine, but not [^{3}H]thymidine, was incorporated into the nucleic acid post-exposure. Since SSB are a consequence of depurination/depyrimidination followed by specific excision of the apurinic-/apyrimidinic-"nucleotide" and guanine is the major alkylation site in DNA exposed to BCES, it was considered that repair was occurring by "base replacement" of guanine residues rather than excision of nucleotide sequences. To confirm this hypothesis, human keratinocytes (HK) in submerged 3 day old cultures were exposed to 1.0 μM BCES and assayed for the incorporation of[^{3}H]guanosine (GdR) (as a measure of "base replacement") or [^{3}H]thymidine (TdR) (to monitor excision repair) into the parental strands of DNA. To seperate the DNA from the RNA, an oligo(dT) cellulose column was used. To distinguish between incorporation as a result of replication and repair, bromodeoxyuridine (BUdR), a heavy analog of TdR, and a cesium chloride gradient were used. After exposure, [^{3}H]TdR was not found in the position of the parental chain. Low levels were found in the position of replicated DNA. [^{3}H]GdR was found in both the parental and relicated position. An enhanced incorporation of [^{3}H]GdR into RNA was seen after exposure. These data are consistent with the hypothesis that the DNA in HK exposed to low levels of mustard compounds is repaired via a guanine specific base replacement rather than excision repair. (Supported by USAMRICD Contract No. DAMD 17–90-C-0031 and ARO Grant No. DAAH04-93-G-0095)

1186 UNEXPECTED DESTRUCTION OF GUANINE RESIDUES IN DNA EXPOSED TO BIS(2-CHLOROETHYL) SULFIDE (BCES)

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75% or more of guanine residues in [^{3}H]guanosine-labelled human keratinocyte(HK) DNA are damaged (i.e., absorbance is lost) after exposure to as little as 50 μM BCES (30'at 37°C). At 50 μM exposure, 90% of these damaged residues retain a normal glycosidic linkage in the DNA. However, at 5000 μM exposure only 25% do so. Isolated HK-[^{3}H]DNA was exposed to 50–5000 μM BCES, hydrolyzed under mild acidic conditions (70°C, pH3.5,30') and then eluted from a column of BioRad P2 to separate large M.W. DNA fragments from expected adducts. These large DNA fragments were susequently treated in 1N $HClO_4$ at 100°C for 60' and eluted on a BioRad P2 column to determine the amount of [^{3}H]guanine residues remaining. In Unexposed HK-[^{3}H]DNA, glycosidic linkages for guanine residues are stable at pH3.5, 70°C and are retained with DNA on P2. These linkages are labile in 1N $HClO_4$, 100°C and elute from P2 as coincident absorbance and radioactive peaks which have been identified on HPLC as [^{3}H]guanine. Exposed HK-[^{3}H]DNA subject to 70°C, pH3.5 dose-responsively elutes a radioactive peak but with no coincident absorbance from P2. Further treatment of the large M.W. DNA fractions (1N $HClO_4$, 100°C) shows a single peak of radioactivity from P2 at the position of [^{3}H]guanine in unexposed DNA but with no coincident absorbance peak. These data suggest the existence of BCES mediated alteration of guanine residues in addition to previously reported alkylated guanine derivatives. Supported by USAMRICD, Contract No.DAMD 17-90-C-0031 and ARO Grant No.DAAL03–92-G-0074.

1187 A MUTAGENICITY ASSESSMENT OF WOODSMOKE-IMPACTED AMBIENT AIR SAMPLES USING THE PLATE AND MICROSUSPENSION SALMONELLA ASSAYS

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The study examines the relative merits of a microsuspension assay and the standard plate assay for monitoring the presence of airborne particle-bound mutagens for hazard identification and comparative hazard assessment studies.

Wintertime ambient air collected from Boise, Idaho, USA contained organic matter mutagenic in the *Salmonella* microsuspension and plate incorporation assays. Mutagenicity of the background site samples contributed from 7 to 34 percent of the total airborne mutagenicity. Differences in the results from the primary sites, auxiliary sites, and the background site demonstrate that the particle-bound mutagens were not evenly distributed within the airshed and were more associated with sampling location than with the time of sampling or the bioassay used. This study demonstrated that the bioassy protocol in such studies should depend upon the characteristics of the airshed mutagens and the purpose of the study. For example, the microsuspension assay gave more variable results but was approximately threefold more sensitive than the plate assay. TA100 with the microsuspension assay provided results comparable to those with TA98. This is important because TA100 can also be used to bioassay semivolatile and volatile organics in airsheds. This in turn allows a comparison of the mutagenicity of organics collected by differing methods due to their volatility. Future efforts will attempt to correlate mutagenicity results with other analytical results in order to further develop methods for better characterization of the genotoxicity of ambient air. [This abstract of a proposed presentation does not necessarily reflect EPA policy.]

1188 GENOPROTECTION BY UDP-GLUCURONOSYLTRANSFERASES (UGTs) IN PEROXIDASE-DEPENDENT MICRONUCLEUS INITIATION BY 4-(METHYLNITRO SAMINO) -1 -(3 -PYRIDYL) -1-BUTANONE (NNK) AND BENZO[A]PYRENE (B[a]P)

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UGTs catalyse NNK and B[a]P glucuronidation, which may reduce competing bioactivation and carcinogenesis. UGTs protect against B[a]P-initiated micronuclei and may provide similar genoprotection against NNK. This study evaluated the relative genotoxic potencies of NNK and B[a]P for micronucleus formation, and genoprotection by UGTs, using skin fibroblasts from +/+ UGT-normal, +/j heterozygous and j/j homozygous UGT-deficient Gunn rats. Cells were incubated with superoxide dismutase (SOD, 1680 IU/ml), inhibitors of cytochromes P450 (CYP) [1 mM 1-aminobenzotriazole, ABT] and peroxidases [ABT; 40 μM eicosatetraynoic acid, ETYA], and inducers of CYP1A1/2 [10 nM 2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD] and peroxidases [TCDD; 0.0367 nM interleukin-1 α, IL-1α; 1 μM 12-O-tetradecanoylphorbol-13-acetate, TPA]. In +/+ cells, NNK and B[a]P initiated concentration-dependent, respective maximal 1.7-fold and 2.7-fold increases over DMSO controls in micronucleus formation ($p<0.05$), with NNK being 1.6-fold more genotoxic than B[a]P ($p<0.05$). In both +/j and j/j cells, NNK- and B[a]P-initiated micronuclei were further enhanced by over 2-fold compared to +/+ cells ($p<0.05$). SOD and P450/peroxidase inhibitors decreased both NNK- and B[a]P-initiated micronuclei, while only that initiated by B[a]P was enhanced, by up to 2.4-fold, by inducers ($p<0.05$). These results provide the first evidence that UGTs are genoprotective for NNK, and that peroxidase-catalysed bioactivation and reactive oxygen species may contribute to the genotoxicity of both NNK and B[a]P. (Support: Medical Research Council of Canada)

1189 MECHANISTIC STUDIES ON BENZENE TOXICITY: FORMATION OF DNA-PROTEIN CROSSLINKS INDUCED BY BENZENE IN MICE AND MUCONALDEHYDE IN HL60 CELLS

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Benzene, a ubiquitous environmental pollutant, is a potent hematotoxin and human leukemogen. The toxicity of benzene is attributed to a number of hydroxylated and ring-opened metabolites, however the mechanism(s) by which these metabolites exert their toxicity is unknown. One mechanism of toxicity may be the formation of DNA-protein crosslinks (DNAPC). DNAPC have been demonstrated for a number of carcinogenic and cytotoxic agents, including UV radiation, alkylating agents, formaldehyde, and the metals nickel and chromium. Muconaldehyde, a reactive hematotoxic ring-opened metabolite of benzene, is a diene dialdehyde and hence a multifunctional alkylating agent. We have demonstrated, using a K^+/SDS precipitation assay, that muconaldehyde induces DNAPC *in vitro* in HL60 cells in a time and dose-dependent manner. In HL60 cells incubated for 2 or 4 hr with muconaldehyde (50–100 uM), DNAPC levels were 2.3 to 5.6, and 2.3 to 7.3 times higher than in controls at the 2 and 4 hr time point, respectively. There was an inverse relationship between HL60 cell viability and DNAPC formation, suggesting a possible contribution of DNAPC formation to cell cytotoxicity. *In vivo*, preliminary results indicate increased levels of DNAPC relative to controls in the bone marrow cells of CD-1 male mice administered hematotoxic doses of benzene (880 mg/kg, 2X/d, 2d). The formation of DNAPC in mouse bone marrow was demonstrated as early as 4 hr following a single ip injection of 440 mg/kg benzene. These in *vitro* and *in vivo* findings suggest that the hematotoxicity of benzene may, in part, be mediated by DNAPC induced by muconaldehyde. Supported by NIEHS Grant ESO2558 and NIEHS Center Grant ESO5022.

1190 GENOTOXICITY TESTS WITH 7-ACETYL-1,1,3,4,4,6-HEXAMETHYL-1,2,3,4-TETRAHYDRONAPHTHALENE (AHTN)

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AHTN, a synthetic tetralin type musk fragrance that has been found in river water and fish, was evaluated for potential genotoxicity in a battery of short-term tests. An *in vitro* cytogenetics assay in Chinese hamster ovary cells was conducted without S-9 activation at concentrations ranging from 3.0 to 23.4 μg/ml using 20 and 44 hour exposure periods and at doses ranging from 5.9 to 78.0 μg/ml during a fourhour exposure period with a 20 hour harvest. In the S-9 activated study, dose levels ranging from 7.8 to 31.2 μg/ml were evaluated for the fourhour exposure period with a 20 hour cell harvest time and at dose levels ranging form 1.0 to 15.6 μg/ml for the fourhour exposure period with a 44 hour cell harvest. No significant increases in structural or numerical chromosome aberrations were observed in the non-activated test system using 4, 20 or 44 hour exposure periods. In the activated portion of the assay, no significant increases in structural chromosome aberrations were observed in the fourhour exposure period with the 44 hour cell harvest. A sight but statistically significant increase in numerical chromosome aberrations was observed in the S-9 activated fourhour exposure period (44 hour cell harvest); the increase was within the range of the historical solvent control. A statistically significant increase in structural chromosome aberrations was observed in the S-9 activated 20 hour cell harvest at all doses tested; the increase was within the range of the historical untreated control at the highest dose level tested and at the remaining dose levels was only slightly outside the historical range for the untreated control. No dose response was observed. AHTN was therefore concluded to be negative for structural and numerical chromosome aberrations in the *in vitro* mammalian cytogenetics test. An *in vitro* unscheduled DNA synthesis (UDS) assay was conducted in primary rat hepatocytes at concentrations between 0.15 and 50 μg/ml (50 μg/ml was too toxic to be evaluated). No increases in net nuclear grain counts were noted. AHTN did not show genotoxic potential based on the negative results in the *in vitro* cytogenetics and *in vitro* UDS assays.

1191 DNA-PROTEIN CROSSLINK FORMATION BY BENZENE METABOLITES IN A CHEMICAL MODEL SYSTEM

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Benzene toxicity is believed to be mediated by its reactive hydroxylated and ring-opened metabolites. One mechanism of toxicity may involve formation of DNA-protein crosslinks (DNAPC). To examine the crosslinking potential of benzene metabolites, a chemical model system was established in which E. coli DNA(100ug/ml) and lysine rich histones (50ug/ml) were incubated with benzene metabolites for 16hr at 37°C. Analysis of DNAPC was carried out using a K+/SDS precipitation assay. Trans, trans-muconaldehyde (muconaldehyde, CHO-M-CHO), a hematotoxic six carbon diene dialdeyde metabolite of benzene, produced dose-dependent DNAPC ranging from 6.3%–13.0% at concentrations ranging from 25–100uM, respectively, compared with 1.0% DNAPC for control DNA-histone samples. The muconaldehyde metabolites 6-hydroxy-trans, trans-2, 4-hexadienal (CHO-M-OH) and 6-oxo-trans, trans-2, 4-hexadienoc acid (CHO-M-COOH) at 100uM also caused formation of DNAPC. The levels of DNAPC decreased in the order CHO-M-CHO > CHO-M-OH > CHO-M-COOH and correlated with alkylating potential. Benzoquinone (BQ) (25-100uM) produced DNAPC in dose-dependent manner similiar to that exhibited by muconaldehyde. The polyhydroxylated metabolites at 100uM produced DNAPC in the order hydroquinone > catechol > 1, 2, 4 benzenetriol. Phenol produced no DNAPC. The formation

of DNAPC by muconaldehyde and BQ could be due to their activity as mulitfunctional direct-alkylating agents. The mechanism(s) of DNAPC formation for the hydroxylated metabolites is unknown. DNAPC produced by benzene metabolites could potentially play a role in benzene hematotoxicity. Supported by NIEHS Grant ESO2558 and NIEHS Center Grant ESO5022.

1192 CHARACTERIZATION OF N¹-ADENOSINE AND N¹-INOSINE ADDUCTS OF BUTADIENE MONOXIDE

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We have previously shown that butadiene monoxide (BM) reacts *in vitro* with nucleosides to form N^7-, N^2-, and N^1-guanosine adducts, and $2N^6$-adenosine adducts. In this study, further characterization of the reaction between BM and adenosine has allowed identification of six additional BM-adenosine adducts. Four of the additional adducts were determined to be diastereomeric pairs of N^1-(1-hydroxy-3-buten-2-yl)adenosine and N^1-(2-hydroxy-3-buten-1-yl)adenosine based on their UV maxima and their ability to undergo the Dimroth rearrangement to yield the corresponding N^6-adenosine adducts. Two additional products of the reaction between BM and adenosine were identified as the diastereomeric pair of N^1-(1-hydroxy-3-buten-2-yl)inosine. This was based on coelution with two of the products of the reaction between BM and inosine, UV spectra, and the fact that N^1-alkyladenosine adducts containing terminal alkyl hydroxyl groups are known to enhance the deamination of the adenosine moiety to inosine. Upon the reaction of BM (200mM) with adenosine (10 mM) in phosphate buffer (pH 7.4) at 37°C, the N^1-adenosine and N^1-inosine isomers were the first to form, with the N^6-adenosine adducts showing a lag in formation due to the time involved in interconversion. After extraction of excess unreacted BM, incubation at 80°C for one hour resulted in complete conversion of N^1-adenosine adducts to the corresponding N^6-adenosine adducts, resulting in the formation of N^6-adenosine and N^1-inosine adducts in a ratio of 9:1. Adduct formation under decreasing BM concentrations resulted in a roughly linear dependency on BM concentration for all six adducts measured. (Supported by NIH grant ESO6841 and an NSF Graduate Research Fellowship).

1193 MICRONUCLEUS TESTS IN MICE WITH HYDROGEN PEROXIDE

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Little information is available concerning the *in vivo* genotoxic potential of H_2O_2, whereas a large amount of data attests to its *in vitro* genotoxic potential. Two micronucleus tests were performed in mice either by intraperitoneal injection or drinking water administration. In the acute i.p. study, groups of Swiss OF1 mice (5/sex/group) were given H_2O_2 once at dose levels of 0, 250, 500 and 1000 mg/kg (1000 mg/kg is the MTD). The drinking water study was a part of a subacute toxicity study (see Weiner et al. in this meeting). C57BL/6N mice (10/sex/group) were given distilled water containing 0 and 6000 ppm H_2O_2 for 14 days (mean daily intakes were 536 and 774 mg/kg bw/d for male and female mice, respectively). In both studies, cyclophosphamide was used as positive control (5 mice/sex). Twenty-four and 48 hours after the acute i.p. administration, and on day 14 of the subacute oral study the animals were killed and bone marrow smears were prepared. For each animal, the micronucleated polychromatic erythrocytes (MPE) were counted in at least 1000 polychromatic erythrocytes (PE) and the polychromatic and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes. In both studies, cyclophosphamide induced a highly significant increase in the number of MPE. After i.p. administration, the PE/NE ratio was significantly lower than the vehicle control group, at the 3 doses of the 24-hour harvest and at 250 and 1000 mg/kg at the 48-hour, showing that H_2O_2 effectively influenced the bone marrow, a similar effect was not observed after the oral administration. In both cases, H_2O_2 did not induce a significant increase in the frequency of MPE. In these studies, H_2O_2 was clearly negative for micronucleus induction.

1194 TOREMIFENE, UNLIKE TAMOXIFEN, IS NOT GENOTOXIC IN RAT LIVER

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Tamoxifen is a potent hepatocarcinogen in the rat, but toremifene, a structural analogue is not. Analysis of rat liver DNA by ^{32}P-postlabelling has shown that large numbers of DNA adducts are formed following tamoxifen treatment, but not toremifene. Rats have been treated with ^{14}C labelled tamoxifen and toremifene, and their liver DNA analysed by ^{32}P-postlabelling, HPLC and accelerator mass spectrometry. An HPLC system has been developed which shows that ^{14}C does not elute with rat liver nucleotides or tamoxifen but elutes in the region of the gradient associated with tamoxifen-DNA adducts. No ^{14}C was detected in this region from toremifene treated rat liver DNA. Unlike tamoxifen, long term treatment with toremifene does not cause hepatocyte hyperplasia as determined by PCNA immunocytochemistry. These results suggest that toremifene is non-carcinogenic because it is unable to act as an initiator of DNA damage, or as a promoter.

1195 GENOTOXICITY TESTS WITH 1,3,4,6,7,8-HEXAHYDRO-4,6,6,7,8,8-HEXAMETHYLCYCLOPENTA-γ-2-BENZOPYRAN

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HHCB, a synthetic musk fragrance that has been found in river water and fish, was evaluated for potential genotoxicity in a battery of short-term tests. A *Salmonella/Escherichia coli* plate incorporation mutagenicity assay was conducted using tester strains TA98, TA100, TA1535, TA1537, TA1538 and WP2 *uvr*A in the presence and absence of Aroclor induced rat liver S-9 at doses from 10 to 5000 μg per plate. In the mutagenicity and confirmatory assays, no positive responses were observed. An *in vitro* cytogenetics assay in Chinese hamster ovary cells was conducted at concentrations ranging from 5.0 to 78.0 μg/ml in the non-activated portion using 4, 20 and 44 hour exposure periods. In the S-9 activated study, dose levels ranging from 2.2 to 69.0 μg/ml were evaluated for the four hour exposure period with a 20 hour cell harvest time and at dose levels ranging form 18.1 to 35.3 μg/ml for the four hour exposure period with a 44 hour cell harvest time. No significant increases in structural or numerical chromosome aberrations were observed in the non-activated test system using 4, 20 or 44 hour exposure periods. In the activated portion of the assay, no significant increases in structural or numerical chromosome aberrations were observed in the four hour exposure period with the 20 or 44 hour cell harvest. HHCB was therefore concluded to be negative for structural and numerical chromosome aberrations in this test. An *in vitro* unscheduled DNA synthesis (UDS) assay was conducted in primary rat hepatocytes at concentrations between 0.15 and 50 μg/ml (50 μg/ml was too toxic to be evaluated). No increases in net nuclear grain counts were noted. HHCB did not show genotoxic potential based on the negative results in the *in vitro Salmonella* and *Escherichia coli* mutagenicity, *in vitro* cytogenetics and *in vitro* UDS assays.

1196 EFFECTS OF ETHANOL ON DNA ADDUCT FORMATION BY URETHANE IN RATS

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Urethane (ethyl carbamate) is an animal carcinogen found at parts per billion levels in alcoholic beverages and fermented foods. The effects of urethane alone and in combination with ethanol on liver DNA adduct formation were investigated in rats. Fisher-344 male rats were exposed to 10,000 ppm of urethane in drinking water and in 5% ethanol for up to 90 days. DNA was extracted from liver, purified and acid-hydrolyzed. DNA hydrolysates were analyzed by high-performance liquid chromatography with UV and fluorescence detection. 7-[2'-Oxoethyl] guanine (OEG) and 1,N^6-ethenoadenine (EA) were found in DNA from rats exposed to urethane alone or in combination with 5% ethanol. The identification of OEG and EA was confirmed by coelution with authentic standards. Neither OEG nor EA was found in rats exposed to 5% ethanol alone. Forty and 67% of rats showed OEG and EA at 2 and 90 days of exposure to urethane in drinking water, respectively. Zero and 10% of rats showed OEG and EA at 2 and 14 days of exposure

to urethane in 5% ethanol, respectively. These results indicate that urethane is capable of forming DNA adducts under drinking exposure conditions. Although the data are preliminary, it appears that, in rats, ethanol may reduce or attenuate formation of DNA adducts by urethane. This finding may have important consequences in that human cancer risk from exposure to urethane in alcoholic beverages may be lower than expected. Further research is needed to confirm or refute this finding.

1197 THE INFLUENCE OF CELLULAR APOPTOTIC RESPONSE ON THE MUTATION FREQUENCY AND SPECTRUM INDUCED BY N-NITROSODIMETHYLAMINE

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N-nitrosodimethylamine (NDMA) is produced in quantities of up to 1 μg/cigarette, and is more predominant in sidestream than mainstream tobacco smoke. Mutational specificity information for NDMA may enable the evaluation of sidestream smoke in the etiology of tumors in passive smokers. The mutagencity of NDMA was investigated in two closely related human cells lines, AHH-1 and MCL-5, which express plasmid transfected cytochrome P450 activities. Although the mutant fraction induced by NDMA at the X-linked *hprt* locus was of the same magnitude in MCL-5 and AHH-1, the induced mutant fraction at the autosomal, heterozygous *tk* locus was 6-fold higher in AHH-1. Analysis of 65 NDMA induced TK. mutants demonstrated qualitative and quantitative differences between MCL-5 and AHH-1 in the spectrum of TK. mutants. Loss of heterozygosity (LOH) was observed in 63% of AHH-1 TK. mutants, but only 14% of those derived from MCL-5. In addition, mapping of LOH tracts using chromosome 17q microsatellite polymorphisms demonstrated that LOH was much more extensive among TK. mutants derived from AHH-1. In fact the entire difference in mutant fraction at the *tk* locus was attributable to large scale LOH events. Analysis of the kinetics of apoptosis in AHH-1 and MCL-5 following exposure to NDMA, suggests that the differences in the recovery of large LOH events is due to the absence of apoptosis in AHH-1 cells. DNA sequence analysis of NDMA induced point mutations will also be presented.

1198 LACK OF DETECTABLE MUTAGENICITY AT THE *LACI* TRANSGENE IN BIG BLUE® RAT EMBRYO FIBROBLASTS FOLLOWING EXPOSURE TO 1,2,3,4-DIEPOXYBUTANE

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1,3-Butadiene and two of its metabolites, 1,2-epoxybutene and 1,2,3,4-diepoxybutane (DEB), are mutagenic *in vitro* and *in vivo*. We have been investigating the mutagenicity of DEB *in vitro* in Rat2 lambda/*lac I* transgenic fibroblasts (Stratagene, LaJolla, CA). Mutagenicity experiments were carried out at concentrations of 0, 2, 5, or 10 μM DEB for 24 hours. These exposure concentrations resulted in approximately 100, 50, and 10% survival respectively. Ethylnitrosourea (ENU), a prototype alkylating agent, was used as a positive control. Cells remained in culture for 72 hours after the DEB was removed, at which time they were trypsinized, pelleted, and frozen for subsequent DNA extraction. Genomic DNA was extracted from cells of each treatment group and assayed for *lacI* mutant frequency. Unexposed controls had a background mutant frequency of $5 \pm 2 \times 10^{-5}$, while cells exposed to 2, 5 or 10 μM DEB had little or no increase over background (5 ± 1, 6 ± 3, and $9 \pm 1 \times 10^{-5}$ respectively). The positive control, 10 mM ENU, induced a mutant frequency at *lacI* approximately 30-fold above background. The increase at the highest exposure concentration of DEB was not significant by Student's *t*-test ($P \leq 0.05$) when compared to media controls. The lack of DEB mutagenicity in these cells may be due to the poor recovery of deletions at the *lacI* transgene. We are examining the induction of micronuclei under the same conditions to assess the role of chromosomal alterations relative to the induction of point mutations to establish a genotoxicity profile for DEB. *Work supported in part by the Health Effects Institute agreement 94–8.*

1199 *IN VITRO* EFFECTS OF AMMONIUM DINITRAMIDE

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Ammonium Dinitramide (ADN) is a high energy compound under study as a replacement for current rocket propellants. This study determined the basic *in vitro* cytotoxicity, stress gene induction, and genotoxicity of ADN. First, liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured to estimate membrane integrity of WB 344 hepatocytes. ADN increased leakage of both these enzymes at all concentrations studied. Normalized EC_{50}s were determined as percentage of controls: ALT EC_{50} = 2.7mM and AST EC_{50} = 3.2mM. Next, to measure interactions of ADN with cellular regulatory transcription factors, genetically engineered human cell lines with fused human and bacterial stress-inducible genes were observed for the stress gene induction followed by ADN treatment. The stress reporter gene induction profile reflected that ADN induced the promoter sequences for all genes observed in the assay. Finally, assays to determine genotoxicity/mutagenicity of ADN were performed. These studies measured ADN's capability of damaging DNA, potentially giving rise to mutations and subsequent tumors. ADN exposed yeast cells indicate that ADN has potential for directly affecting intrachromosomal recombination. This study is a vital part of the initial phase of toxicity testing and evaluation of ADN.

1200 THE GENOTOXICITY OF NICKEL COMPOUNDS DEPENDS ON NICKEL SPECIATION AND EXPOSURE CONDITIONS

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This study examined how nickel speciation could influence the genotoxicity of various nickel compounds in liver, kidneys and peripheral lymphocytes of mice and rats in vivo. The genotoxicity was measured as DNA damage in the form of double-strand DNA breaks (ds DNA breaks) which was evaluated by the fluorometric assay of DNA unwinding. As a positive control, methylmethane sulfonate was used. Adult male CD-1 mice or Sprague-Dawley rats were treated subcutaneously (s.c.), or intraperitoneally (i.p.) or orally (po) with either Ni_3S_2 or $NiCl_2$ or $NiSO_4$ (0, 0.03, 0.06, 0.12 and 0.25 mmole/kg) or NiO(0, 0.20, 0.40, 0.80 mmole/kg), only once, or once a day for 3 consecutive days. 24 hours following exposure the animals were sacrificed. Significant induction of ds DNA breaks were observed in the kidneys of mice treated s.c. at 0.25 mmole/kg of Ni_3S_2, in both the liver and kidneys of mice at 0.06 mmole/kg, i.p. and only in the liver at 0.25 mmole/kg, p.o. No such strand-breaks were observed in rats. $NiCl_2$ induced such DNA strand-breaks in both the liver (30%) and kidneys (49%) of mice but only in the rat liver at 0.25 mmole/kg, i.p. $NiSO_4$ induced such damage in mice kidneys only when treated s.c. In contrast, NiO failed to modify ds DNA in rats or mice. None of these compounds induced any DNA strand-breaks in peripheral lymphocytes. Pretreatment of mice with Vitamin C or Vitamin E reduced significantly the induction of ds DNA breaks in the kidneys due to Ni_3S_2, suggesting the involvement of free radicals in such toxicity. Thus the extent of induction of ds DNA breaks depends not only on the nickel speciation but also on the exposure route and animal species. (Supported by Health Canada)

1201 UDP-GLUCURONOSYLTRANSFERASE (UGT)-MEDIATED GENOPROTECTION IN PHENYTOIN- AND 5-(p-HYDROXYPHENYL)-5-PHENYLHYDANTOIN (HPPH)-INITIATED DNA OXIDATION AND MICRONUCLEUS FORMATION

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The DNA-damaging teratogen phenytoin is eliminated primarily by hydroxylation to HPPH, a supposedly non-teratogenic metabolite eliminated by UGT-catalysed glucuronidation. This study evaluated whether phenytoin and HPPH were genotoxic (DNA oxidation, micronucleus formation), and whether UGTs were genoprotective by reducing competing bioactivation of HPPH to a reactive free radical intermediate. Skin fibroblasts from +/+ UGT-normal, and +/j heterozygous and j/j homozygous UGT-deficient Gunn rats were incubated with either 80 μM phenytoin or 10 or 80 μM HPPH. Micronucleus formation initiated by both phenytoin and HPPH was increased by a minimum of 1.7-fold in +/+ cells and a maximum of 4.8-fold in j/j cells versus DMSO controls ($p \leq 0.03$), with a minimum 2.0-fold increase in UGT-deficient versus normal cells ($p \leq 0.02$). HPPH 10 μM did not initiate micronuclei in +/+ cells, but produced a maximal 4.0-fold increase in UGT-deficient cells versus DMSO controls ($p = 0.0001$). DNA oxidation measured by high-performance liquid chromatography with electrochemical detection was increased by 80 μM HPPH in j/j cells, but was not detectable in either untreated or HPPH-treated +/+ cells ($p < 0.05$). These results provide the first evidence that both HPPH and phenytoin are genotoxic, and suggest that HPPH-initiated

reactive oxygen species may contribute to phenytoin teratogenicity, particularly in women with hereditary UGT-deficiencies. (Support: Medical Research Council of Canada)

1202 POLYCYCLIC AROMATIC HYDROCARBON-DNA ADDUCTS IN EXFOLIATED UROTHELIAL CELLS AND URINARY 1-HYDROXYPYRENE IN ROAD PAVING WORKERS

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Million of tons of asphalt are used every year, mainly in road construction and maintenance. The carcinogenic potential of asphalt and asphalt fumes is a major concern of the public due to the presence of polycyclic aromatic hydrocarbons (PAH). No data are available on exposure of road construction workers or on effects of carcinogens during road paving in the U.S. In order to evaluate road paving workers occupationally exposed to PAH in asphalt, a pilot study using biomarkers, 1-hydroxypyrene in urine and PAH-DNA adducts in exfoliated urothelial cells was conducted. 1-Hydroxypyrene was measured by high-performance liquid chromatography with fluorescence detection. PAH-DNA adducts were measured by ^{32}P-postlabelling. The study is in progress. The samples analyzed show to date a wide variability in 1-hydroxypyrene (84.34nmole/L to undetected).

1203 METABOLISM OF ESTRADIOL IN THREE DIFFERENT BREAST CELL LINES

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The mechanism of estrogen carcinogenesis is not yet fully understood. Recent evidence has shown that levels of estrogen-catechol metabolites may play a critical role in oxidative DNA damage in breast tissue and that estrogen metabolism to these catechols may be modified by environmental xenobiotics. In order to understand how estrogen metabolism may contribute to oxidative damage, we have studied the metabolism of estradiol (E2) in three different breast cell lines: MCF10A, a non-tumorigenic cell line, MCF7 and MDA MB231, two tumorigenic breast cell lines. Each cell line was pre-incubated ± TCDD, a CYP4501 family inducer, for 72h. After the addition of [^{3}H]E2, formation of ethyl acetate extractable metabolites was determined at 1,3,6, and 24h. Without TCDD, MCF7 and MDA MB231 metabolized only 45% [^{3}H]E2 by 24h. In contrast, MCF10A metabolized 60% of the [^{3}H]E2 by 3h and 80% by 24h. In the presence of TCDD, only MCF7 cells at 24h had an appreciable increase in E2 metabolism, from 45% without TCDD to 78% with TCDD. Inducibility of E2 metabolizing enzymes at the molecular level is also being investigated in the three cell types. These results suggest that MCF10A, MCF7, and MDA MB231 differentially metabolize E2 and that each responds differently to TCDD. Therefore, further use of these cells to study variations in oxidative DNA damage due to metabolic differences is merited. Supported by ES07141, ES03819, and CA06973.

1204 HIGH LEVELS OF ALKYLGUANINE ALKYLTRANSFERASE, AND OF O^6-METHYLGUANINE DNA ADDUCT IN EXTRA-HEPATIC TISSUES AFTER EXPOSURE OF MONKEYS TO N-NITROSODIMETHYLAMINE

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The ubiquitous environmental carcinogen, N-nitrosodimethylamine (NDMA) has been suspected as contributing to causation of human head/neck and gastrointestinal tract cancers. However, it does not cause high levels of DNA adducts or tumors in these tissues of rodents. To investigate the susceptibility of primate tissues, we administered NDMA (0.1 mg/kg ig) to three female and two male patas monkeys. Thirty-two types of tissue were taken 4 hrs later and tested for the methylation of guanine in DNA at the O^6 position by a sensitive competitive-repair assay. High levels of this promutagenic adduct, comparable to those in liver (1.8 ± 0.3μmol/molG), occurred in several tissues: gastric mucosa (2.2 ± 0.7); esophagus, colon, white blood cells, pancreas, urinary bladder, ovary, and uterus (0.8–1.2 μmol/molG). Lowest levels were in lung (0.1 μmol/molG). The repair enzyme alkylguanine alkyltransferase (AGT) which was assayed in parallel varied over a 30-fold range, with highest levels in liver (47 fmol/μg DNA) and stomach (38 fmol/μg). Activities of 19–20 fmol/μg were found in pancreas and small and large intestine, and of 10–19 fmol/μg in esophagus, kidney, ovary, spleen, and peripheral lung. Lowest activity was in gray matter of brain (1.5 fmol/μg). Thus, some extrahepatic primate tissues have capacity to form and to repair O^6-methylguanine to an extent similar to that of liver, and hence may be targets for carcinogenesis by NDMA.

1205 QUANTITATION OF N^2,3-ETHENOGUANINE (£G) IN RAT LIVER BY LIQUID CHROMATOGRAPHY ELECTROSPRAY MASS SPECTROMETRY

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Gas chromatography mass spectrometry (GC-MS) has provided both the sensitivity and specificity needed for the quantitation of N^2,3-ethenoguanine (£G, a highly promutagenic DNA adduct produced *in vivo* from vinyl chloride (VC) exposure. This method requires chemical derivatization followed by clean-up prior to analysis. Due to low yields or analytical interferences arising from the derivatization procedure, sample quantitation can be ambiguous. The objective of this research was to determine if £G quantitation can be performed using liquid chromatography electrospray mass spectrometry (LC-ESMS) without derivatization. £G and spiked internal standard $^{13}C_4$-£G were isolated from DNA hydrolysates by low pressure chromatography (strong cation exchange and C-18 solid phase extraction). To evaluate the potential of LC-ESMS, £G was measured in calf thymus DNA alkylated with chloroethylene oxide (CEO) using SIM at m/z = 176 (£G) and m/z = 180 ($^{13}C_4$-£G). The amount of £G was 30.1 ± 4.5 pmol/mgDNA (n = 14), demonstrating good precision. Two or four hrs after exposure, the amounts of £G in liver DNA from 2 Sprague-Dawley rats exposed to 0.29 μmol CEO/g body weight via the portal vein was 1.38 ± 0.07 (n = 4) and 0.39 ± 0.04 (n = 4) pmol/mgDNA, respectively. These preliminary data demonstrate that LC-ESMS can be used to quantify £G in exposed animals. (Supported by NIEHS grant P42-ESO594803)

1206 MOLECULAR DOSIMETRY OF N^2,3-ETHENOGUANINE IN DNA OF VINYL FLUORIDE-EXPOSED RATS: RELATIONSHIP TO HEMANGIOSARCOMA

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A recently completed carcinogenicity bioassay on vinyl fluoride (VF) demonstrated the induction of hemangiosarcomas of the liver in rats and mice, with a supralinear exposure-response believed to be due to saturation of metabolic activation. Liver samples from a 12 month interim sacrifice were collected and DNA was analyzed for N^2, 3-ethenoguanine (EG) using GC/high resolution MS. Steady-state concentrations of EG exhibited a supralinear exposure-response in both species, with 1.24 ± 0.31, 2.24 ± 0.96, and 1.93 ± 0.43 pmol EG/μmol G in mice exposed to 25, 250, or 2500 ppm VF and 0.70 ± 0.28, 0.95 ± 0.22, and 0.94 ± 0.20 pmol EG/μmol G in rats. Unexposed mice and rats had endogenously formed EG present at 0.54 ± 0.21 and 0.17 ± 0.05 pmol EG/μmol G, respectively. When the the molecular dose of EG was compared with the incidence of hemangiosarcomas in all exposure groups, a linear relationship was evident ($r^2 = 0.88$). Furthermore, when EG was measured in hepatocytes and nonparenchymal cells (NPC) of rats exposed to 2500 ppm VF for 4 weeks, the NPC had 1.22 ± 0.89 pmol EG/μmol G, while the hepatocytes had 0.47 ± 0.18. These data demonstrate the utility of molecular dosimetry for identifying factors responsible for nonlinearities in carcinogenic response. In addition, the clear demonstration of endogenous EG raises important questions related to the extent of risk associated with low environmental exposure to vinyl halides. (Supported by grants from DuPont and NIEHS P42 ESO5948.)

1207 CHARACTERIZATION OF THE MAJOR PROPYLENE OXIDE GUANINE ADDUCT AND THE DEVELOPMENT OF A METHOD FOR ITS DETECTION BY HIGH RESOLUTION MASS SPECTROMETRY

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Propylene oxide (PO) is classified as a possible human carcinogen. It is a relatively weak mutagen that causes nasal tumors in rats at concentrations that also cause increases in cell proliferation. In order to more accurately assess the carcinogenic risk of PO, it is necessary to understand the relationships between DNA damage, cell proliferation and neoplasia. The objective of this research is to characterize the DNA adducts formed by propylene oxide. Initially, the reaction of propylene oxide with guanosine was studied. HPLC coupled with diode array detection was employed to separate and purify the adducts formed. Propylene oxide reacted as an S_N2 alkylating agent, leading to the formation of a major adduct which was identified as N-7-(2-hydroxypropyl)guanine (7HPG). The adduct was characterized by UV spectroscopy, NMR, and electrospray mass spectrometry. 7HPG was also the major DNA adduct formed following exposure of calf-thymus DNA to propylene oxide *in vitro*. An electrophoric derivative of this adduct was prepared in order to be detected with high sensitivity by gas chromatography. The tris-pentafluorobenzyl product was obtained by converting the adduct to the corresponding xanthine, and then reacting the residue with pentafluorobenzylbromide. This product was analyzed by gas chromatography high resolution mass spectrometry using selected ion monitoring at m/z 569.0671, which corresponds to loss of one pentafluorobenzyl group from the parental anion radical. This method will be used to determine the molecular dose of 7HPG in DNA from tissues of animals exposed to propylene oxide by the inhalation route. (Supported by NIEHS grant T32-ESO712611 and a grant from the Chemical Manufacturers Association)

1208 HEPATIC AFLATOXIN B_1 METABOLISM IN THE MARMOSET CALLITHRIX JACCHUS

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It is well established that certain species such as the mouse are resistant to the hepatocarcinogenic effects of aflatoxin B_1 (AFB_1), whereas other species such as the rat are highly sensitive. Previous studies have shown that AFB_1 metabolism in humans differs significantly from that in rodents, therefore, data obtained from rodent studies cannot be readily extrapolated to humans. In order to find out whether non-human primates represent a suitable model for human AFB_1-metabolism, we carried out a study investigating *in vitro* phase I and phase II AFB1-metabolism in the liver of the marmoset monkey *Callithrix jacchus*. Considering the inter-individual variation in the production of AFB_1 oxidative-metabolites, hepatic microsomes from 3 individual marmosets and 5 humans generated similar amounts of AFB_1-8,9-epoxide (AFBO) as well as the hydroxylation products AFM_1 and AFQ_1, at both low and high substrate concentrations (15,25 μM, 126 μM). Furthermore, in contrast to rodents, marmoset and human liver cytosols did not exhibit detectable AFBO-glutathione conjugating activity. The results of this *in vitro* study suggest that the marmoset may be a useful model for studying AFB_1 metabolism in humans. (Supported by ES-05780-08).

1209 LACK OF DNA ADDUCT FORMATION BY SULFASALAZINE (SASP) IN LIVER AND URINARY BLADDER OF MICE AND RATS

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SASP has been used for over 40 years in the treatment of inflammatory bowel diseases and rheumatoid arthritis. Increases of sister chromatid exchange and micronuclei (MN) have been reported in lymphocytes from patients treated with SASP and MN in RBC from mice treated with SASP. Long-term carcinogenicity studies indicated an increase of liver tumors in mice and of urinary bladder (UB) tumors in rats given SASP. The potential of SASP to induce DNA adduct formation in the liver and UB was evaluated, when given at doses of up to 1000 and 3000 mg/kg/d for 7 days to male and female rats and mice, respectively. The ^{32}P postlabeling procedure with P_1 enhancement was used. Positive controls were 2-AAF and 2-naphthylamine for the liver and UB. Radioactive spots indicative of I-compounds were present in the autoradiograms from both tissues in both species. The positive control spots were present in the target tissues. However, no radioactive spots attributable to SASP-DNA adducts were found in either liver or UB in rats or mice. These results support other recent findings suggesting that the genotoxic and tumorigenic effects of SASP in rodents are due to epigenetic factors such as interference with folate uptake or topical stimulation and cytotoxicity, which have no correspondence in man.

1210 FORMATION OF EPOXIDE-INDUCED 3-HYDROXYALKYL-URACIL IN DNA: MECHANISTIC STUDIES

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3-Hydroxyalkyl-deoxyuridine (3-HA-dU) is induced in DNA by mutagenic and carcinogenic aliphatic epoxides such as ethylene oxide (EO) and propylene oxide (PO). It is formed after initial alkylation at N3 of deoxycytidine (dC) followed by rapid (< 10 h) hydrolytic deamination. We have previously shown that 3-HA-dU can be mutagenically bypassed during *in vitro* DNA replication. This study investigated the mechanism of formation of EO- and PO-induced 3-HA-dU. We have postulated that the hydroxyl group on the alkyl side chain attacks a positively charged carbon at C4 of 3-HA-dC, which causes deamination and following hydrolysis the formation of 3-HA-dU. We have confirmed this mechanism by determining that 3-HA-dC has a pKa of 8.7, and that the hydroxyl group speeds up the deamination 60-fold. We determined that the reaction is base catalyzed and goes through a cyclic intermediate. We have isolated and spectroscopically characterized the cyclic intermediate and determined the kinetics of hydrolytic deamination at the base level. We have demonstrated that the hydrolysis occurs at C4 by the use of $H_2{}^{18}O$ and mass spectral analysis. This study suggests that 3-HA-dU can be formed in DNA by environmentally important epoxides and may contribute to mutagenesis and the initiation of cancer. Supported by grants from NIEHS (ESO5694) and NIH Center grants ESO0260 and CA16087.

1211 FORMATION OF BUTADIENE MONO- AND DIEPOXIDE DNA ADDUCTS IN CALF THYMUS DNA

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Butadiene (BD) is a widely used industrial chemical, environmental pollutant and a possible human carcinogen. BD is weakly tumorigenic in rats (≥1000 ppm) and is a potent carcinogen in mice (≥6.25 ppm). Epoxybutene (EB) and diepoxybutane (DEB) are thought to be the ultimate reactive metabolites of BD. Species differences in sensitivity to BD are likely to be due to the higher formation of DEB in mice compared to rats. DEB is a bifunctional alkylating agent with a potential of forming multiple adducts and cross-links. In this work, the major adducts induced by DEB and EB in calf thymus DNA were identified using HPLC with UV detection and LC/ESMS in selected ion monitoring mode. Calf thymus DNA (36 mg) was reacted with 4.7 mmol EB or DEB in 1:1 mixture of 10 mM buffer and methanol at 37°C for 18 hours. The adducts were released by neutral thermal or mild acid hydrolysis and subjected to HPLC analysis after microconcentration. An additional desalting step using C18 SPE columns was necessary prior to LC/MS analysis. Adduct identification was based on their co-elution with authentic standards on two HPLC columns, UV spectra from diode array detection and molecular weight information from MS. The N-7 position of guanine was the most reactive in both cases, with N7-(2-hydroxy-3-buten-1-yl) guanine, N7-(1-hydroxy-3-buten-2-yl)guanine and N-7-(2,3,4-trihydroxybutyl) guanine being the predeominant adducts formed. EB was more reactive then DEB, suggesting that the higher mutagenicity of DEB may be due to its bifunctional properties. (Supported in part by a grant from the Chemical Manufacturers Association)

1212 AGE-DEPENDENT MODULATION OF TISSUE-SPECIFIC REPAIR ACTIVITY FOR METHYLPURINE GLYCOSYLASE

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The ability to repair promutagenic damage resulting from exposure to carcinogens, i.e. vinyl chloride (VC), may be a critical factor in determinig the target cell for neoplasia. One major pathway for the repair of chemically induced DNA damage involves base-excision repair by N-methylpurine-DNA-glycosylase (MPG). We have measured expression levels of MPG in liver and

lungs of Sprague-Dawley rats as a function of age. A quantitative reverse transcriptasepolymerase chain reaction (QRT-PCR) method was used for measuring cellular MPG mRNA. The efficiency of the reverse transcription was monitored in all reactions by the addition of an internal reference RNA, which contained a single base mutation creating an Eco R1 restriction site. Primers specific for cellular MPG were radiolabelled and used to amplify a 471 base pair fragment. PCR products were electrophoresed on a polyacrylamide gel and the radioactive bands excised and quantitated by liquid scintillation counting. Initial data in rats (n = 2-3/age group) show that preweanling and adult liver yield 0.57 ± .34 and 0.92 ± .43 (pg/μg total RNA), respectively and preweanling and adult lungs yield 1.79 ± .87 and 1.02 ± .67 (pg/μg total RNA), respectively. It is clear that MPG levels are tissue - and age-dependent, being greatest in preweanling lung. Additional tissues with and without VC exposure will be analyzed to further evaluate the role of DNA repair in VC carcinogenicity. (Supported in part by NIEHS grant P42-ESO5948).

1213 RELATIONSHIP BETWEEN 1,N^6-ETHENODEOXYADENOSINE (εA) FORMATION AND MUTATIONS AT A-T BASEPAIRS IN VINYL CHLORIDE- AND CHLOROETHYLENE OXIDE-EXPOSED HUMAN CELLS

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It has been previously demonstrated that vinyl chloride (VC) and its reactive metabolite chloroethylene oxide (CEO) produce similar mutational spectra at the *hprt* locus, with a high incidence (43/62) of base substitutions at AT sites. This suggests that εA (or its corresponding apurinic site) is an important DNA adduct. In this study the molecular nature of 15 additional independent splicing aberration mutants was determined by sequence analysis of the missing exons and flanking intron regions amplified from genomic DNA in human B-lymphoblastoid cell lines expressing rat (R2E1) or human (H2E1) cytochrome P450 2E1. One mutant had a single base deletion; all the others contained base pair substitutions, with about 60 % at AT sites. Thus, 51/76 *hprt* mutations induced by VC and CEO were at AT sites. εA was analyzed using immunoaffinity/^{32}P-postlabeling in cells exposed to 75,000 ppm VC, an exposure inducing a 6–8 fold increase in *hprt* mutants. Significant amounts of εA were found in R2E1 and H2E1 cells, with yields of 4.9 ± 1.2 x 10^{-8} and 8.5 ± 4.9 x 10^{-8}, respectively. In addition, exposure to an equi-mutagenic dose (20 μM) of CEO resulted in 4.3 ± 1.2 x 10^{-8} of εA. εA was also present in unexposed cells at 0.6 ± 0.3 x 10^{-8}. The increases in mutations and the relative amount of εA present after VC and CEO exposure suggest that εA may be the promutagenic lesion responsible for the VC-induced mutations at AT sites observed in 2E1-expressing human culture cells. (Supported by grants from NIEHS P42-ESO5948 and El DuPont Co.)

1214 DETECTION OF DNA ADDUCTS IN COAL MINERS EXPOSED TO DIESEL EMISSIONS IN AUSTRALIA

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Diesel operated engines are used in Australian coal mining operations. The recognised carcinogens, polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs, have been identified in diesel engine emissions (DEE) and their potential long term health hazards have been of increasing concern. An early crucial step in chemical-induced carcinogenesis is considered to be formation of adducts between the chemical and DNA. The technique of ^{32}P-postlabelling offers the possibility of detecting DNA adducts at a high level of sensitivity in workers exposed to such carcinogens. Workers who were exposed to DEE in underground coal mining in Australia were invited to participate in the study. For 61 workers from one mine, blood samples were collected both before and after a long wall change out operation, which is a period of heavier exposure. Peripheral blood mononuclear cells were isolated and the DNA adducts were measured by the ^{32}P-postlabelling technique. The same plate was used for the two samples (before and after) for each worker. The use of the same plate to analyse samples from the same worker was an advantage as it removed the plate to plate variability in the assay. The results show that geometric mean of total DNA adducts was significantly increased (297 vs 389 amol/μg DNA, $p<0.0001$, paired t test) after the period of increased exposure. Some individual adducts were also elevated to a greater extent ($p<0.05$, paired nonparametric test). However, comparison of the low, medium and high exposure groups (categorised by questionnaire) did not demonstrate any significant differences. This study demonstrates that DEE causes a significant increase in total and some individual DNA adducts in humans after a period of heavier exposure.

1215 *IN VITRO* REACTIONS OF HYDRAZINE WITH DEOXYRIBONUCLEOSIDES

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The National Aeronautics and Space Administration uses hydrazine (Hz) to propel the Space Shuttle and satellites, and plans to use it aboard the International Space Station as well. Hz is also used by the U.S. Armed Forces. Hz vapor, a strong irritant, has induced nasal tumors in chronically exposed rats and hamsters. Reactions of DNA with anhydrous Hz at elevated temperature have been observed, but direct reactions under physiological conditions have not been reported. This preliminary study was conducted in an effort to elucidate the possible mechanism of Hz carcinogenesis. Deoxyriboxynucleosides (dG, dA, dT, dC) were incubated (37°C) with phosphate buffered Hz solutions (0.5 mM or 2.5 mM, pH 7.4) for up to 24 hr. The reaction mixtures were analyzed by HPLC. All the bases reacted with Hz; the amount of reaction products increased with time. The reactions of dA with Hz were further studied in the presence of iron salts or iron-containing biomacromolecules. The results indicated that iron enhanced the reactions and catalase was the most active iron-containing compound in inducing the degradation of the base. The HPLC results suggested that Hz reacted with dC to form products including N^4-aminocytidine, a suspected mutagen. Studies are planned to use a triple-stage quadrupole mass spectrometer coupled with an atmospheric pressure ionization (API) ion source and an HPLC to positively identify the Hz-nucleoside reaction products.

1216 ^{32}P-POSTLABELING ASSAY FOR DETECTION OF ADDUCT FORMATION BY ZOPOLRESTAT (CP-73,850) IN RAT LIVER DNA

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Carcinogens and mutagens covalently bind DNA bases and can be detected by a ^{32}P-postlabeling assay. Zopolrestat, [4-dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl] methyl]-1-phthalazinylacetic acid] was delivered to male and female Sprague-Dawley rats daily by gavage for 7 days at 0, 125 or 250 mg/kg bw. The positive control 7, 12-Dimethylbenz [a] anthracene (DMBA) was delivered by gavage as a single dose of 60 mg/kg bw 24 hours before the sacrifice. Rats were sacrificed 24 hours following the last dose administration and livers were harvested. DNA from the livers was extracted and processed for ^{32}P-postlabeling using the nuclease P_1 enhancement procedure. There were neither deaths nor any other compound related observations. A total of four (4) I-spots (naturally occurring indigenous modified bases) were observed in both treated and untreated rats. The positive control DMBA demonstrated one adduct spot in addition to the I-spots. However, there were no modified bases (DNA adducts) other than I-spots in the Zopolrestat treated rat livers. Thus, Zopolrestat was shown not to produce DNA adducts in liver DNA when administered to rats.

1217 CHARACTERIZATION OF DNA ADDUCTS FOLLOWING *IN VITRO* REACTION TO TETRANITROMETHANE (TNM) BY CAPILLARY ELECTROPHORESIS/ MASS SPECTROMETRY (CE/MS)

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Biomonitoring exposure to environmental and occupational carcinogens is often based on measurements of carcinogen-DNA adducts. Characterization of such adducts is also valuable for determining reaction mechanisms which result in potentially harmful DNA adducts. Identification and measurement of such adducts has become possible with the development of the sensitive and specific analytical technique of CE/MS without the use of radiolabeling. In the present study, DNA and nucleobase standards were reacted *in vitro* with the potent pulmonary carcinogen TNM at 37° for 24 hours. DNA was hydrolyzed to normal and adducted bases which were separated and analyzed by CE/MS using 50 mM ammonium acetate and 50 mM acetic acid using an 80 cm x 50 mm untreated fused silica column at 30 kV (>200,000

theoretical plates). The CE column was introduced into an electrospray probe with added isopropanol/water/acetic acid (4mL/min) to provide electrical contact. DNA adducts increased for each base in the order of guanine > adenine > cytosine>thymine. Adducts identified included guanine + $C(NO_2)$, guanine + NO_2 and guanine +NO. These results demonstrate the utility of CE/MS for analysis in DNA adduct research.

1218 CHARACTERIZATION OF 4-AMINOBIPHENYL HEMOGLOBIN ADDUCTS IN MATERNAL AND FETAL BLOOD SAMPLES

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The maternal-fetal exchange of the potent tobacco related human carcinogen, 4-aminobiphenyl, was studied in women smokers during pregnancy. The number of cigarettes smoked per day by each of the women in the study was assessed via questionnaire and by measurement by immunoassay of serum and urine cotinine in maternal and fetal blood samples. Maternal and fetal blood samples were classified as coming from nonsmokers (n = 74), individuals smoking less than 1 pack of cigarettes per day (n = 16), individuals smoking 1 pack of cigarettes per day (n = 19), individuals smoking 1–2 packs of cigarettes per day (n = 19), and individuals smoking greater than 2 packs of cigarettes per day (n = 20). Both maternal and fetal blood samples were obtained at the time of delivery. 4-Aminobiphenyl was extracted from both maternal and fetal blood samples using organic extractions and the released amine was qualitatively and quantitatively characterized by analysis of the samples by gas chromatographic and mass spectrometric analysis. Background levels of 4-aminobiphenyl - hemoglobin adducts were detected in maternal nonsmokers (18.3 ± 12.7 pg 4-aminobiphenyl / g hemoglobin, mean ± SD) and in fetal samples (8.88 ± 5.8 pg / g hemoglobin). Increasing levels of 4-aminobiphenyl - hemoglobin adducts were found as the smoking status of the women increased ranging from 144 ± 22.2 (<1 pack per day) to 633 ± 87.9 (>2 packs per day). A corresponding increase in the presence of fetal 4-aminobiphenyl hemoglobin adducts was also detected (74.3 ± 17.8; <1 pack/day to 319 ± 50.5; >2 packs/day). This study confirms that the potent tobacco related carcinogen, 4-aminobiphenyl, crosses the human placenta and binds to fetal hemoglobin in significantly higher concentrations in smokers when compared to nonsmokers.

1219 QUANTITATIVE ANALYSIS OF BENZO(A)PYRENE HEMOGLOBIN (Hb) ADDUCTS IN MATERNAL AND FETAL BLOOD OBTAINED FROM SMOKERS AND NONSMOKERS

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The maternal-fetal exchange of the tobacco related carcinogen, benzo(a)pyrene, was studied in women smokers during pregnancy. The number of cigarettes smoked per day by each of the women in the study was assessed via questionnaire and by measurement by immunoassay of serum and urine cotinine in maternal and fetal blood samples. Maternal and fetal blood samples were classified as coming from nonsmokers (n = 74), individuals smoking less than 1 pack of cigarettes per day (n = 16), individuals smoking 1 pack of cigarettes per day (n = 19), individuals smoking 1–2 packs of cigarettes per day (n = 19), and individuals smoking greater than 2 packs of cigarettes per day (n = 20). Both maternal and fetal blood samples were obtained at the time of delivery. After lysing the red cells with chilled deionized water, the globin was precipitated using acidified acetone. Paired globin samples were hydrolyzed under acidic conditions to remove the bound benzo(a)pyrene from the protein. Benzo(a)pyrene tetrols were isolated using a C18 Sep-Pak cartridge eluted with water and methanol. Tetrols were evaporated to dryness under nitrogen and stored at −20° C until analysis by HPLC and GC/MS. Background levels of benzo(a)pyrene - Hb adducts were detected in maternal nonsmokers (6.83 ± 3.46 pmol BP tetrol / g Hb, mean ± SD) and in fetal samples (3.46 ± 1.81 pmol / g Hb). Increasing levels of benzo(a)pyrene - Hb adducts were found as the smoking status of the women increased ranging from 18.93 ± 5.21 (<1 pack per day) to 96.75 ± 18.66 (>2 packs per day). A corresponding increase in the presence of fetal benzo(a)pyrene Hb adducts was also detected (10.31 ± 3.11; <1 pack/day to 52.55 ± 14.26; >2 packs/day). This study confirms that the tobacco related carcinogen, benzo(a)pyrene, crosses the human placenta and binds to fetal Hb in significantly higher concentrations in smokers when compared to nonsmokers.

1220 ACRYLONITRILE OXIDATION AND CYANIDE RELEASE IN CELL FREE SYSTEM CATALYZED BY FENTON-LIKE REACTION

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Acrylonitrile (VCN) induces human and animal gastrointestinal cancer. The present study was designed to investigate if reactive oxygen species (ROS) similar to that generated in biological systems are capable of oxidative activation of VCN molecules and subsequent cyanide (CN^-) release. Initial studies utilized a model ROS generating system (Fenton-Like Reaction) that contained Fe^{+2} and H_2O_2 and predominantly produces hydroxyl radical (·OH). The current results indicate that VCN was markedly oxidized by this system. The rate of VCN oxidation is depend on VCN concentration. Increasing H_2O_2 concentration (50 to 200 μmoles) resulted in a 50-fold increase in CN^- release. Studies on the effect of various transition metals on the rate of oxidation of VCN revealed that the rate of CN^- formation was highest with ferrous (Fe^{+2}) followed by copper (Cu^{+1}) and lowest with titanium (Ti^{IV}). Addition of various concentrations of ROS scavengers; DMSO, mannitol, sodium formate, or sodium benzoate to the incubation mixtures resulted in a significant and a concentration dependent decrease in CN^- release. Addition of iron chelators; desferrioxamine (DFO) or 2,3 dihydroxy benzoic acid (DHB) to the incubation mixtures produced a significant decrease in CN^- release. Catalase, when added to the incubation mixtures induced the highest rate of inhibition of VCN oxidation to CN^-. Lineweaver-Burk and Michaelis-Menten analysis of the kinetic parameters indicated that catalase was the strongest inhibitor for ROS mediated oxidation of VCN to CN^- (K_i = 0.03 mM) followed by DFO (K_i = 0.07 mM), mannitol (0.13 mM), DMSO (0.15 mM), sodium formate (0.23 mM), sodium benzoate (0.24 mM), and DHB (0.33mM). In conclusion, our results indicte that VCN is metabolized by ROS mediated mechanism in cell free system. This mechanism may play a role in VCN-induced genotoxicity. [Supported by NIEHS grant No. ES 01871]

1221 A ROLE OF REACTIVE OXYGEN SPECIES IN THE PATHOGENESIS OF OF ACRYLONITRILE INDUCED GASTRIC MUCOSAL CELL DAMAGE

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Acrylonitrile (VCN) causes acute gastric hemorrhage and mucosal erosion. The objective of the present study is to investigate the role of oxygen radicals in VCN-induced damage to cultured gastric mucosal cells (GMCs). Freshly cultured GMCs were used in these studies. Cytotoxicity was quantitated by D-^{14}C-glucose release from prelabeled cells. Cell death was measured by crystal violet uptake and thiazolyl blue (MTT) reduction. VCN caused a time and dose-dependent increase in D-^{14}C-glucose release. Exposure of GMCs to VCN resulted in a dose dependent decline of the intracellular GSH contents (up to 50% of the control) after 60 min incubation. GMCs exposed to various concentration of VCN produced increasing levels of superoxide anion (O_2.), as assessed by cytochrome c reduction. Exposure of GMCs to VCN induced increase of plasma membrane damage, as measured by leakage of lactate dehydrogenase (160% of control). Mitochondrial succinate dehydrogenase activity was decreased (55% of control) following exposure of GMCs to VCN. Pretreatment with N-acetyl-L-cysteine (NAC; 1-3mM) protected against VCN-induced D-^{14}C-glucose release from prelabeled GMCS (40% of control). Incubation of GMCs with superoxide dismutase (SOD)[5-500 U/ml) and catalase (3–3000 U/ml) decreased VCN-iduced cytotoxicity (up to 73% and 58% of control respectively). Diethylcarbamate (0.1-0.3mM) and 3-aminotriazole (1-3mM) specific inhibitors of SOD and catalase, respectively, enhanced VCN-induced cytotoxicity. In conclusion, our results suggest that ractive oxygen species are essential mediators in VCN-induced GMCs damage. NAC, SOD and catalase play a critical role as cellular antioxidant defence against VCN-induced cellular damage in cultured rat GMCs. [Supported by NIEHS Grant No. ES 01817].

1222 ADRIAMYCIN INDUCED HEPATIC OXIDATIVE INJURY AND ANTIOXIDATIVE RESPONSES IN MALE AND FEMALE MICE

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The toxicity of Adriamycin (ADR), a major limiting factor for clinical use of this important anticancer agent, is believed to be mediated by production of reactive oxygen species during its intracellular metabolism. Cardiotoxicity of ADR has been extensively studied, but little is known about its hepatotoxic-

ity. Therefore, the present study was undertaken to determine the effect of ADR on the liver, focusing on oxidative injury and antioxidative defense. Male and female FVB mice were treated with ADR at 20 mg/kg by a single ip injection. Four days after the treatment lipid peroxidation in the liver was measured. The results showed that ADR elevated lipid peroxide levels (by 46%, $p<0.05$) selectively in the male mouse liver. To determine the possible mechanism(s) for the sex difference in response to ADR, alterations of hepatic antioxidant systems by ADR were compared between male and female mice. ADR induced 5-fold increase in metallothionein (MT) concentrations in both male and female mouse liver. This induction was corresponding to the elevation of mRNA levels. Superoxide dismutase (SOD) and glutathione reductase (GR) activities were not changed in either male or female liver. The activities of glutathione peroxidase (GSH-Px) and catalase were inhibited by 31% and 44%, respectively, in the male mouse liver ($p<0.05$). The activity of γ-glutamylcysteine synthetase (pigg-GCS) was depressed by 36% ($p<0.05$) also only in the male mouse liver, with a corresponding 36% decrease in the hepatic glutathione (GSH) content. Northern blot analysis revealed that ADR suppresses expression of mRNAs for SOD, catalase, and γ-GCS in the male liver. These findings demonstrate that differential oxidative injuries induced by ADR exist in male and female mouse liver, which may result from sex-related alterations in regulation of antioxidant systems. Supported in part by NIH Grants CA68125 and CA63752.

1223 EXTRACELLULAR OXIDATIVE STRESS DURING ALL-TRANS-RETINOL POTENIATION OF CARBON TETRACHLORIDE (CCl_4) HEPATOTOXICITY IN THE MALE SPRAGUE-DAWLEY RAT

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Previous studies evaluating retinol (vitamin A) potentiation of carbon tetrachloride hepatotoxicity have suggested that retinol enhances CCl_4 induced lipid peroxidation by increasing hepatic oxidative stress. Furthermore, studies have shown that retinol potentiated hepatotoxicity can be blocked by quenching reactive oxygen species (ROS) using PEG-SOD. The objective of this study was to evaluate the role of ROS in retinol potentiation of CCl_4 by monitoring blood and biliary glutathione (GSH) and glutathione disulfide (GSSG) levels as an index of oxidative stress. Control and retinol pretreated male Sprague-Dawley rats (75 mg/kg/d for 7d) were anesthetized with urethane. Following catheterization of the carotid artery or bile duct, CCl_4 (200 μl/kg, *i.p.*) was administered. Liver tissue, arterial blood, and bile were collected at various time points (0 to 24h) and evaluated for GSH and GSSG by HLPC. Neither retinol nor CCl_4 altered the basal blood concentrations of GSH or GSSG. However, when CCl_4 was administered to retinol pretreated animals a 2–3 fold increase in blood GSSG was observed up to 4 hr after CCl_4 administration. Following CCl_4 administration, biliary excretion of GSH increased between 0 and 1 hr and then declined significantly (1 to 4 hr) in both vehicle and retinol pretreated rats. Interestingly, no increases in the content of either biliary GSSG or liver tissue GSSG were observed. From these data, we conclude that extracellular oxidative stress occurs during retinol potentiation of CCl_4, and that the likely source of this oxidative stress is the release of reactive oxygen species by Kupffer cells and other phagocytic cells. (Supported in part by P30 ES 06694, RO1-ES-06095, and a P&G Graduate Fellowship).

1224 MUTATIONS IN pSP189 TREATED WITH PEROXYNITRITE OR ACTIVE OXYGEN SPECIES AND REPLICATED IN BACTERIAL AND HUMAN CELLS

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Chronic infections are risk factors for certain cancers such as *Helicobacter pylori* infection and gastric cancer, and hepatitis B virus infection and liver cancer. Reaction of nitric oxide with superoxide produced by inflammatory cells may form highly reactive peroxynitrite (ONOO.), a potent mutagen. Mutation spectra induced by ONOO. were characterized in the shuttle vector pSP189 after *in vitro* exposure and replication in bacterial or human cells. Virtually all base-substitution mutations occurred at G:C base pairs, with fewer than 1% occurring at A:T base pairs. Predominant mutations were G:C T:A transversions (65% and 63% after replication in bacterial and human cells, respectively); G:C C:G transversions were present at a lower frequency (28% and 11%). G:C T:A transversions can result from the presence of 8-hydroxyguanine residues as well as other oxidatively damaged bases in replicating DNA, suggesting that ONOO. may have induced mutations through oxidative damage to DNA. Molecular mechanisms of ONOO.-mediated DNA damage are not known, but possible pathways include H^+-catalyzed decomposition to form an intermediate with the reactivity of OH·, and/or metal-catalyzed formation of a nitronium-like nitrosating species (NO_2^+). Previous studies of mutations induced by OH· reported primarily G:C A:T transitions as well as significant amounts of base substitutions at A:T base pairs. In order to compare the mutation spectra induced in pSP189 by ONOO. to those induced by OH., we treated the vector with γ-irradiation prior to replication in bacterial or human cells. Comparative findings will be presented. (Supported by NIH 2-PO1-CA26731).

1225 EFFECTS OF VITAMIN E SUCCINATE (VES) ON NAPHTHALENE-INDUCED HEPATIC AND BRAIN LIPID PEROXIDATION, DNA SINGLE STRAND BREAKS, GLUTATHIONE DEPLETION, MEMBRANE MICROVISCOSITY AND EXCRETION OF URINARY LIPID METABOLITES IN RATS

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Naphthalene (NAP) causes oxidative damage in hepatic and brain tissues. Therefore, the effects of VES on NAP-induced oxidative stress and tissue were examined. Female Sprague-Dawley rats were treated with a single dose of 1100 mg NAP/kg (0.50 LD_{50}). VES treated rats received 100 mg VES/kg/day for 3 days before NAP treatment, and 40 mg VES/kg/day after NAP treatment. Hepatic and brain tissues and urine were collected 0, 12, 24, 48, and 72 hrs after NAP treatment. NAP treatment resulted in a 2.1-fold increase in LP in liver and brain mitochondria at 24 hrs. Increases in hepatic and brain mitochondrial LP in VES plus NAP treated rats were 39–46% less than in NAP treated rats at 24 hrs. DNA-SSB increased 3.0-fold in hepatic tissues in NAP treated rats, and increased only 1.6-fold in VES protected rats at the 24 hrs. GSH decreased by 83% and 49% in hepatic and brain tissues, respectively, in NAP treated rats at 24 hrs, while GSH in VES plus NAP treated rats was 43% and 58% less in hepatic and brain tissues, respectively. Microsomal MF, a measurement of membrane damage, increased 1.9- and 1.7-fold in liver and brain tissue, respectively, in NAP treated rats, and only 1.3- and 1.2-fold in NAP plus VES treated rats at 24 hrs. The urinary excretion of malondialdehyde (MDA), formaldehyde (FA), acetaldehyde (ACT), and acetone (ACON) was determined at 0–96 hrs after NAP administration. Between 12–24 hrs after NAP treatment maximal excretion of these four ULM was observed, with increases of 4.5-, 2.7-, 2.3-, and 2.8-fold for MDA, FA, ACT, and ACON, respectively, at 24 hrs. VES reduced the NAP induced excretion of these urinary metabolites by 28–49% 24 hrs after NAP treatment. These results support the hypothesis that NAP induces oxidative tissue damage, and VES provides significant protection.

1226 THE ANTIOXIDANT FUNCTION OF THE PHYSIOLOGICAL CONTENT OF VITAMIN C IN CULTURED ODS HEPATOCYTES

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Osteogenic Disorder Shinogi (ODS) rats are unable to synthesize vitamin C. Hepatocytes prepared from ODS rats had less than 10% of the wild type content of this vitamin. In culture, the ascorbate content remained low in the absence of supplementation. Independent of their vitamin C status, cultured ODS hepatocytes become depleted of vitamin E. Supplementation of the culture medium with 100 μM ascorbate and 1.2 μM α-tocopherol phosphate maintained the physiological content of both vitamin C and E. Thus, the antioxidant function of vitamin C and vitamin E could be evaluated in the presence of both or either vitamin, or in the absence of both vitamins. Hepatocytes deficient in both vitamins were the most susceptible to the peroxidation of membrane lipids and to cell killing within a 90 minute exposure to 125–500 μM *tert*-butyl hydroperoxide (TBHP). Supplementation to achieve a physiologic content of both ascorbate and α-T reduced the evidence of lipid peroxidation and abolished the cell killing. Supplementation with either vitamin alone resulted in an intermediate degree of both lipid peroxidation and cell killing. In ODS hepatocytes treated with TBHP, the decline in vitamin E preceded the decline in vitamin C. In ODS hepatocytes depleted of vitamin C, the loss of vitamin E following exposure to TBHP was greater than that in the presence of physiological levels of ascorbate. This greater loss of vitamin E in the face of a depletion of vitamin C was

readily attributable to the increased peroxidation of lipids. By contrast, the rate and extent of the depletion of vitamin C correlates with the degree of cell killing. These data document of the physiological content of vitamin C and relate this function to protection against peroxidative cell injury. In addition, the physiological content of vitamin C in cells does not appear to regenerate vitamin E.

1227 COMPUTER-AIDED DOSE-RESPONSE CHARACTERISTICS OF CHEMICALLY INITIATED OXIDATIVE STRESS IN VITRO

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A computerized, biologically based pharmacodynamic (BBPD) model was developed to simulate the dose-dependent biological effects of chemically induced oxidative stress in tissue preparation *in vitro*, based on description by Vroegop et al. (Free Radical Biol. Med. 18, 141, 1995). The model was written in Advanced Continuous Simulation Language (ACSL) with a FORTRAN sub-routine, and simulations were performed using SIMUSOLV software with optimization capabilities (DOW Chemical Co., Midland, MI) on a VAX/VMS mainframe computer. The BBPD model simulated formation of free radicals over time as a function of the chemical inducer, and predicted the distribution of dose-dependent inhibition of cellular receptors at each time. The BBPD model was validated with the literature data for generation of free radicals in liver slices (Steel-Goodwin et al. Toxicologist 15, 30(161), 1995), and for dose-dependent effects of oxidative stress in cultured neuroma cells (Vroegop et al. 1995). The BBPD model allowed us to distinguish the "one hit" targeted mode of action of free radicals, inhibiting specific cellular receptor, from "nonspecific" interaction with pleiotropic receptors. It is suggested that the algorithm developed and calibrated with experimental data *in vitro* may be employed for future dose-response characterization of action of prooxidant chemicals *in vivo*, using the physiologically based pharmacokinetic/dynamic model.

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1228 EFFECT OF TIRILAZAD MESYLATE (U-74006F) ON LIPID PEROXIDATION (LPO) AND MEMBRANE STABILIZATION IN HEPATIC INJURY MODELS

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The hypothesis was tested that U-74006F protects against liver injury by inhibition of LPO or by cell membrane stabilization. In male Fischer rats subjected to 20 min of hepatic ischemia followed by reperfusion and injection of 0.5 mg/kg *Salmonella enteritidis* endotoxin, development of liver injury was accompanied by LPO, as indicated by 81–184% increases of hepatic 8-, 9-, 11-, and 12-hydroxy eicosatetraenoic acid (HETE) content and a 85% increase of plasma F_2-isoprostane concentrations at 4 h of reperfusion. Pretreatment with U-74006F (6 mg/kg) reduced hepatic injury by 60% but had no significant effect on either parameter of LPO. In contrast, U-74006F treatment attenuated liver injury and lipid peroxidation at 24 h reperfusion. Pretreatment with U-74006F *in vivo* had no effect on LPO and injury in the isolated perfused liver during perfusion with 500μM *tert*-butyl hydroperoxide. However, U-74006F protected hepatocytes significantly against membrane damage induced by cell swelling due to perfusion with hypotonic medium. These data support the conclusion that U-74006F enhances the resistance of liver cell membranes to injury predominantly by its membrane-stabilizing effect and not by directly scavenging free radicals. U-74006F may inhibit LPO as a secondary effect due to its modification of membrane properties.

1229 SUPPRESSION OF OXIDATIVE TOXICITY IN THE CATALASE OVEREXPRESSING HEART OF TRANSGENIC MICE

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Catalase is a major enzyme involved in detoxification of hydrogen peroxide. The activity of catalase per g tissue in the heart is very low, being only about 2% that of liver in the mouse. This less proficient ability to dispose of reactive oxygen species may be responsible for the high sensitivity of this organ to oxidative injury. The objective of this research is to determine whether elevation of catalase activity specifically in the heart of transgenic mice can provide protection against oxidative cardiotoxicity. Multiple transgenic mouse lines in which catalase is specifically overexpressed in the heart have been produced. Each transgenic line exhibits a stably elevated cardiac catalase activity, ranging from 2-to 500-fold higher than normal. This enzyme activity is not altered in other organs including liver, lung, kidney, and skeletal muscles. Other antioxidant systems are not changed in the heart of transgenic mice. This unique experimental model was used to determine the effect of varying catalase activities on cardiotoxicity induced by Adriamycin, hydrogen peroxide, and anoxia-reoxygenation. Measurements of lipid peroxidation, serum creatine phosphokinase activity and atrial function demonstrated that transgenic mouse hearts overexpressing catalase activity 50- to 100-fold higher than normal exhibited marked resistance to cardiac damage under the oxidative conditions described above. This study provides direct support for the hypothesis that augmentation of cardiac antioxidative mechanism provides protection against oxidative injury to the heart. Supported in part by NIH Grants CA68125 and CA63752, and A Grant-In-Aid from the American Heart Association 94007070.

1230 L-DOPA-INDUCED OXIDATIVE STRESS IN THE MONKEY STRIATUM

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Increased turnover of dopamine has been suggested to contribute to the degeneration of nigrostriatal neurons in Parkinson's disease *via* the MAO-dependent production of hydrogen peroxide. To test this hypothesis, dopamine turnover was stimulated in the striatum of squirrel monkeys by administration of the dopamine precursor L-dopa (40 mg/kg) together with the peripheral decarboxylase inhibitor benserazide (12.5 mg/kg). Oxidized glutathione (GSSG) was measured as an indicator of hydrogen peroxide accumulation. L-dopa treatment caused a 6-fold increase in dopamine turnover as assessed by the ratio DOPAC/dopamine. This increase was accompanied by a significant elevation of striatal GSSG level and GSSG/GSH ratio. Administration of either the MAO A inhibitor clorgyline (2 mg/kg) or the MAO B inhibitor deprenyl (2 mg/kg) at selective doses prior to L-dopa exposure counteracted the increase in dopamine turnover and completely reversed GSSG elevation. This is the first demonstration of L-dopa-induced oxidative stress in the striatum of non-human primates. Data support the concepts that (i) administration of high doses of L-dopa may affect the survival of dopaminergic neurons in the nigrostriatal pathway of patients with Parkinson's disease, and (ii) MAO inhibitors may protect against these toxic effects of L-dopa.

1231 TCDD, ENDRIN AND LINDANE INDUCED INCREASES IN LIPID METABOLITES IN MATERNAL SERA AND AMNIOTIC FLUIDS OF PREGNANT C57BL/6J AND DBA/2J MICE

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TCDD, endrin and lindane induce an oxidative stress and enhance lipid peroxidation in fetal and placental tissues of mice. The levels of the products resulting from altered lipid metabolism, including malondialdehyde, formaldehyde, acetaldehyde and acetone, in response to these compounds have been determined in maternal sera and amniotic fluids of pregnant C57BL/6J and DBA/2J mice after oral administration of single fetotoxic doses on day 12 of gestation using high pressue liquid chromatography. Under these conditions, TCDD given at a dose of 30 μg/kg body weight to C57BL/6J mice produced 2.5–3.9 and 1.7–4.0 fold increases in the levels of the 4 lipid metabolites in maternal serum and amniotic fluid, respectively. TCDD given to DBA/2J mice at a dose of 60 μg/kg produced 1.5–1.7 and 1.7–2.2 fold increases in the levels of these metabolites in maternal serum and amniotic fluid, respectively. Endrin, given at a dose of 4.5 mg/kg body weight produced increase of 1.4–1.6 and 1.7– 2.4 fold in the levels of the 4 lipid metabolites in maternal serum and amniotic fluid, respectively, in the C57BL/6J, and increases of 1.4–1.6 and 1.2–1.5 fold in the levels of these metabolites in maternal serum and amniotic fluid, respectively, in DBA/2J mice. Lindane given at a dose of 30 mg/kg body weight produced 1.5–2.4 and 1.3–1.7 fold increases in the levels of the 4 lipid metabolites in maternal serum and amniotic fluid, respectively, in C57BL/6J mice, and increases of 1.2– 1.5 and 1.4–1.5 fold in the levels of the metabolites in maternal serum and amniotic fluids, respectively, in DBA/2J mice. The results suggest that increases in the levels of lipid metabolites in maternal serum and amniotic fluid as a result of lipid peroxidation may participate in the fetotoxic effects of TCDD, endrin and

lindane regardless of the Ah-responsiveness, although higher levels are produced by TCDD in Ah-responsive mice.

1232 COCHLEAR PROTECTION FROM FREE RADICALS DURING CARBON MONOXIDE (CO) EXPOSURE

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The proposed mechanism underlying the auditory dysfunction associated with CO exposure involves the generation of free radicals due to hypoxia. Free radical scavengers/inhibitors have been shown to be protective in other organs during hypoxic events such as ischemia/reperfusion and in the ear during noise exposure. This study evaluated the protection afforded by two such agents; PBN which acts as a general free radical scavenger and allopurinol which acts as a free radical inhibitor specific to the xanthine oxidase metabolic pathway. Guinea pigs were exposed to CO (35 ml/kg ip) with and without PBN (100 mg/kg ip) or allopurinol (100 mg/kg ip) administered prior to exposure. They were monitored at 15, 30, and 60 min after CO exposure for elevations in compound action potential threshold. The groups receiving CO alone displayed characteristic threshold elevations particularly at the higher frequencies (16–40 kHz). Of the protected groups, those receiving PBN displayed protection at all time periods. The group receiving allopurinol exhibited a mixed effect with the end result being partial protection at best. The study strengthens the evidence implicating free radical generation during hypoxia associated with CO exposure as the destructive mechanism within the cochlea. The mixed results with allopurinol point to metabolic pathways other than xanthine oxidase as the source of those free radicals. Supported by NIH grant ES02852 and EPA fellowship U-914755-01-0.

1233 OXIDATIVE STRESS IN TOXIC EFFECTS OF REPIN, A SESQUITERPENE LACTONE ISOLATED FROM RUSSIAN KNAPWEED

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Russian Knapweed (*Centaurea repens*) is a perenial weed found in many parts of the world, including Southern California. Ingestion of this plant by the horse has been known to cause nigro-striatal encephalomalacia associated with signs and symptoms similar to those of Parkinson's disease. Repin is one of the principal ingredients isolated from Russian knapweed, and is found to be highly toxic to mice. Previous studies in our laboratory showed that PC12 cells exposed to repin undergo dose-dependent decrease in both cell viablilty and glutathione (GSH) concentration. To further examine the mechanism(s) of toxic effects of repin, PC12 cells were exposed to varying doses (40 and 80 μM) of repin, and the rate of reactive oxygen species (ROS) production was evaluated by the use of dichlorofluorescein diacetate (DCFDA). PC12 cells were preincubated in 5 μM DCFDA for 45 minutes in RPMI 1640 and then exposed to repin in Locke's buffer for 10 minutes. The fluorescence was read at 488 nm (excitation) and 525 nm (emission). The rate of ROS production was markedly increased from the control value of 0.836 fmol/min/cell to 6.809 fmol/min/cell in repin-exposed cells. These results suggest that oxidative stress plays a major role in toxic actions of repin as evidenced by both increased production of ROS and significant decrease in GSH. (Supported in part by NIH grant ES 02928).

1234 AN EMBRYO CULTURE MODEL TO ELUCIDATE MECHANISMS OF STRAIN SUSCEPTIBILITY: PHENYTOIN EMBRYOPATHY IN A/J MICE

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The A/J mouse is the strain most susceptible to phenytoin teratogenesis, which may result from embryonic peroxidase-catalysed bioactivation and reactive oxygen species production. Although in vivo strain differences are well documented, the underlying mechanism of differential susceptibility is unknown, and may be relevant to human susceptibility. This study first evaluated the potential to culture embryos from inbred A/J mice, and then determined whether this strain was susceptible to phenytoin teratogenesis in culture. Embryos were explanted from pregnant A/J dams on gestational days 9.5–10.0 (vaginal plug = day 1) and examined for somite development. Comparable A/J embryonic somite development (4–6 somites) occurred later (day 10.0) than in embryos from outbred CD-1 dams (day 9.5), therefore subsequent A/J embryos were explanted on gestational day 10.0. Explanted embryos were incubated for 24 hr at 37°C in culture medium containing either phenytoin (20 μg/ml, 80 μM) or its vehicle (0.002N NaOH). Vehicle-exposed embryos developed normally compared to the CD-1 strain. A/J embryos incubated with phenytoin had substantial reductions in anterior neuropore closure, turning, yolk sac diameter and somite development compared to controls ($p<0.05$). These results indicate that A/J embryos are capable of phenytoin bioactivation, with embryopathic consequences, and hence may be used as a model to provide insights into the molecular mechanisms of strain susceptibility to xenobiotic teratogenesis. (Supported by the Medical Research Council of Canada)

1235 EFFECTS OF CHRONIC ALCOHOL ADMINISTRATION ON NITRIC OXIDE PRODUCTION BY HEPATIC MACROPHAGES AND ENDOTHELIAL CELLS

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Hepatic macrophages (MP) and endothelial cells (EC) release an array of proinflammatory and cytotoxic mediators that have been implicated in the development of liver injury. In the present studies we analyzed the effects of chronic alcohol administration on production of nitric oxide by these cells. Rats were exposed to ethanol (10.4 g/kg) by intragastric feeding. Cells were isolated from the animals after 3–6 weeks of treatment by *in situ* perfusion of the liver with collagenase and pronase followed by differential centrifugation and elutriation. We found that MP and EC from both untreated and alcohol-treated rats readily synthesized nitric oxide following *in vitro* stimulation with interferon-γ (IFNγ) and lipopolysaccharide (LPS). This response was dependent on l-arginine and was blocked by two nitric oxide synthase inhibitors, N^G-monomethyl-l-arginine and l-canavanine. MP and EC produced similar amounts of nitric oxide in response to these inflammatory mediators. Nitric oxide production in response to LPS plus IFNγ was decreased in both cell types following treatment of rats with alcohol. MP appeared to be more sensitive to the *in vivo* effects of alcohol than were EC. Northern and western blot analysis demonstrated that nitric oxide production by MP and EC was due to increased expression of an inducible form of nitric oxide synthase (iNOS) mRNA and protein. Taken together, our data demonstrate that exposure of rats to alcohol decreases the capacity of both MP and EC to produce nitric oxide. Decreased sensitivity of nonparenchymal cells to inflammatory mediators may be important in the hepatic response to alcohol. (Supported by GM34310 and the Burroughs Wellcome Fund.)

1236 INTERACTION OF DIQUAT AND CYTOKINES IN HEPATOTOXICITY AND NITRIC OXIDE INDUCTION

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The toxicity of diquat (1, 1'-ethylene-2,2'-dipyridylium), a highly redox active bipyridyl compound, can be entirely attributed to the production of oxidative stress. Previous work in this laboratory has demonstrated that the cytotoxicity and gene induction effects of the cytokine tumor necrosis factor-α (TNF-α) are mediated by the production of reactive oxygen intermediates (ROI). The objective of the present study was to explore the hypothesis that the hepatic effects of diquat/TNF-α combinations would be especially pronounced due to the common mechanisms of ROI generation. In primary cultured mouse hepatocytes, diquat was found to cause dose dependent cytotoxicity (as assessed by LDH leakage into the medium), and depletion of cellular glutathione (GSH). The addition of TNF-α to the incubations caused a significant shift in the diquat dose response curve for cytotoxicity, as well as enhanced loss of GSH. Diquat, which generates oxygen radicals intracellularly, did not induce nitric oxide synthase (NOS). Conversely, xanthine/xanthine oxidase, which generates superoxide extracellularly, was found to significantly induce NOS. The data suggest that TNFα and diquat may cause synergistic effects on GSH depletion and hepatotoxicity through their common effects on oxygen radical generation. However, the intracellular generation of ROI by diquat did not induce NOS, suggesting that the location of ROI generation is important for the signalling effects of oxygen radicals on gene induction. Supported by NIH grant DK44755.

1237 THE MITOCHONDRIAL PERMEABILITY TRANSITION CAUSED BY MENADIONE IS INHIBITED BY BENZOQUINONE

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Induction of the mitochondrial permeability transition is implicated in the mechanism of mitochondrial dysfunction and cell injury caused by numerous oxidants. According to one model, the permeability transition pore is regulated by a thiol-dependent voltage-sensor subject to either oxidative or electrophilic modification. The objective of this investigation was to determine whether alkylating quinones (e.g., benzoquinone; BQ) alter the voltage-dependent regulation of the permeability pore in a manner similar to or distinct from that caused by redox cycling quinones, such as menadione (MQ). Freshly isolated rat liver mitochondria were incubated in vitro with 50 μM MQ and/or 6 μM BQ. Membrane potential (Δ¥) was measured using a TPP^+-selective electrode and mitochondrial volume was assessed spectrophoto-metrically. MQ increased the gating potential of the voltage sensor resulting in the induction of the mitochondrial permeability transition. This was prevented by N-ethylmaleimide and reversed by dithiothreitol. Although BQ inhibited NADH-ubiquinone oxidoreductase directly, it did not induce the permeability transition in mitochondria energized with succinate. In fact, pre-incubation with BQ prevented the MQ-induced depolarization and permeabilization of hepatic mitochondria. We concur that the permeability transition pore is regulated by a thiol-dependent voltage sensor. Oxidation by redox cycling quinones to form the disulfide increases the gating potential whereas substitution by electrophilic quinones to form thioethers prevents it.

1238 PROMOTION OF RENAL PROXIMAL TUBULE CELLULAR (RPTC) REGENERATION FOLLOWING OXIDANTINJURY BY ASCORBIC ACID PHOSPHATE

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Exposure of rabbit RPTC to oxidants results in cell death and is followed by a transient regeneration of the monolayer due to cell migration/spreading but not proliferation (TAP 132:273, 1995). Since ascorbic acid has been implicated in tissue growth and repair, we investigated the effect of L-ascorbic acid phosphate (AAP) on the regeneration of RPTC monolayers following exposure to the oxidant t-butylhydroperoxide (TBHP). Rabbit RPTC were grown in improved culture conditions (Am. J. Physiol. 268:C1053, 1995) in the absence or presence of 0.5 mM AAP. AAP addition had no effect on DNA content in control confluent RPTC but increased RPTC oxygen consumption (1.3-fold), net lactate consumption (2-fold), net glucose production (2-fold), Na^+-dependent glucose uptake (1.5-fold), and the activity of the brush border marker γ-glutamyl transpeptidase (1.3-fold). Treatment of confluent RPTC with 0.8 mM TBHP for 50 min decreased confluency and DNA content to 35% and 41%, respectively, after 24 hrs in control and AAP-grown RPTC. In control RPTC, confluency transiently increased to 44% after 72 hrs, but decreased to 31% after 96 hrs. DNA content did not increase between 24 and 96 hrs. In RPTC cultured continuously in the presence of AAP, confluency increased to 76% and DNA increased 3-fold after 96 hrs. These results show that AAP treatment 1) further improves the physiological properties of cultured RPTC by increasing respiration and gluconeogenesis, 2) promotes the recovery of the injured RPTC monolayer in the absence of exogenous growth factors, and 3) stimulates proliferation of RPTC following oxidant injury. (Supported by NIH ES-04410).

1239 DIFFERENTIAL EFFECTS OF SUPEROXIDE DISMUTASE AND U74389G ON TRIMETHYLTIN-INDUCED COCHLEAR DISRUPTION

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Trimethyltin (TMT) is a neurotoxicant believed to induce reactive oxygen species (ROS) generation in neural tissues, and an ototoxicant which disrupts cochlear compound action potential (CAP) thresholds. In order to test whether the ototoxicity of TMT is related to cochlear ROS generation, anesthetized guinea pigs had a large neck vein catheterized and received surgery to expose the middle ear. CAP thresholds were recorded from the round window for ten pure tone acoustic frequencies (2-35 kHz). The animals were then administered iv 0.9% saline, polyethylene glycol-conjugated superoxide dismutase (PEG-SOD, 10,000 U/kg) or the 21-aminosteroid, U74389G (4.5 mg/kg, Upjohn), 30 min prior to an ip injection of TMT-Cl (0.5 mg/kg) or saline. Electrophysiological potentials were recorded over 90 min and converted to pre-post exposure difference scores representing CAP threshold shifts. Control animals receiving PEG-SOD, U74389G or saline followed by saline did not differ significantly from one another in terms of mean CAP threshold changes ($p > .05$), each having relatively stable sensitivity. Saline followed by TMT administration resulted in a rapid, significant ($p < .05$) increase in mean CAP threshold values at all tested frequencies relative to controls. Although PEG-SOD followed by TMT administration resulted in some CAP threshold degradation, these shifts were significantly less than those of the saline-TMT group at all frequencies at 30 min ($p < .05$), and at all frequencies except those between 6 and 12 kHz at 90 min ($p < .05$). Threshold shifts for the group receiving U74389G prior to TMT were not significantly different than saline-TMT group values at any frequency. These results suggest that TMT-induced CAP disruption may be partially due to ROS generation.

1240 NEPHROTOXICITY OF 17β-ESTRADIOL AND CATECHOL ESTROGEN GLUTATHIONE (GSH) CONJUGATES IN SYRIAN HAMSTERS

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Hormones can play an important role in the initiation and promotion of cancer. Hormonal carcinogenesis is often characterized by both tissue and species specificity. For example, male Golden Syrian hamsters, an established model of hormonal carcinogenicity, develop renal carcinoma following prolonged exposure to 17β-estradiol (E_2). The basis for this specificity is unclear. The catechol estrogens have been suggested as putative redox active metabolites contributing to the carcinogenic effect of E_2. Following administration of E_2 (50 μmol/kg, ip) to hamsters, elevations in the urinary excretion of γ-glutamyltranspeptidase (γ-GT), alkaline phosphatase, glutathione-S-transferase and glucose were detected. Protein carbonyls and lipid hydroperoxides, markers of oxidative damage, were also elevated. E_2 toxicity was ameliorated by pretreating hamsters with an inhibitor of γ-GT (AT-125), implying toxicity is mediated by metabolites requiring metabolism by this enzyme. Consistent with this view, administration of 2-hydroxy-1-glutathion-S-yl-E_2 or 2-hydroxy-4-glutathion-S-yl-E_2 (0.27–5.0 μmol/kg) also produced mild nephrotoxicty in Syrian hamsters, an effect that was absent in $B_6C_3F_1$ mice treated in a similar manner. Repeated daily administration of 2-hydroxy-4-glutathionyl-S-yl-E_2 (7 days, 5.0 μmol/kg) resulted in a sustained elevation of urinary markers of renal damage and in renal protein carbonyls and lipid hydroperoxide levels. In summary, we have demonstrated the mild nephrotoxicity of E_2 and catechol estrogens thioethers to hamsters (CA 58036).

1241 PEROXIDE RESISTANCE AND BCL-2 EXPRESSION IN HUMAN TUMOR CELL LINES

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The t(14; 18) translocation present in most B cell lymphomas causes an excess production of the bcl-2 protooncogene protein. Overexpression of this protein has been found to enhance cell survival by inhibiting apoptosis induced by many agents include free radicals. Although the function of the 26 kDa *bcl-2* encoded protein is not known, a link between free radicals, apoptosis and *bcl-2* is supported by several studies. The current study was designed to assess the expression of bcl-2 protein in cell lines selected for resistance to increasing amounts of H_2O_2. Human glioma U87MG cells were selected for resistance to 0, 50, 100, 200 and 400 μM H_2O_2 by exposure to this oxidant for 10 months. When examined 16 days after cessation of peroxide exposure by western blots with peroxidase/diazobenzidine detection, bcl-2 protein levels were increased 45, 49, 86 and 115% with increasing H_2O_2 resistance. The expression of bcl-2 protein at other times after cessation of peroxide exposure, as well as bcl-2 mRNA levels, will be determined. The available data suggest that chronic oxidant exposure yields cells that are resistant to oxidant damage and contain increased levels of bcl-2 protein. Additional studies, including time-dependence and mRNA levels, are needed to establish whether bcl-2 is causally involved in the mechanism(s) of peroxide resistance. This work was supported by grants HL 51005 (JPK) and HL 51469 (DRS).

1242 PROTECTION AGAINST 2, 3, 5-(TRIGLUTATHION-S-YL)-HYDROQUINONE-MEDIATED TOXICITY IN RENAL PROXIMAL TUBULE EPITHELIAL CELLS (LLC-PK_1) BY PROSTAGLANDIN E_2

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Prostaglandins (PG) are cytoprotective in a variety of pathologic conditions, and PGE_2 is rapidly synthesized by LLC-PK_1 cells following exposure to 2, 3, 5-(tri-glutathion-*S*-yl) hydroquinone (2, 3, 5-[triGSyl]HQ). The role of this prostanoid in 2, 3, 5-(triGSyl)HQ-mediated cytotoxicity was investigated. Pretreatment (24 h), but not cotreatment, of cells with PGE_2-methyl ester (10-30 μM) protected against 2, 3, 5-(triGSyl)HQ (300 μM) mediated cytotoxicity. Pretreatment of cells with the prostaglandin EP2 receptor agonist (11-deoxy-16, 16-dimethyl PGE_2; DDM-PGE_2 0.1-20 μM) for 24 hr also prevented the toxicity of 2, 3, 5-(triGSyl)HQ. Maximum protection by DDM-PGE_2 occurred between 50–100 nM. Sulprostone (an EP3 receptor agonist) produced only modest protection, whereas PGE_1, and 17-phenyltrinor PGE_2 (an EP1 receptor agonist) did not afford protection. Gel mobility shift analysis showed that 1 μM DDM-PGE_2 and 12–O-tetradecanoyl phorbol-13-acetate (10 ng/ml; TPA) induced binding to a TPA responsive element (TRE) as early as 0.5 hr, with no significant binding to a cAMP responsive element. Induction of TRE-binding activity by DDM -PGE_2 and TPA, and DDM-PGE_2-mediated cytoprotection, were blocked by the protein kinase inhibitor H-89 (40 μM). Pretreatment (24 h) of cells with TPA (10 ng/ml) also protected against toxicity, whereas forskolin (0.1–30 μM), dibutyryl cAMP (0.0001–10 mM), or the Ca^{2+} ionophore 4-Br-A23187 (0.001–1.0 μM) were without effect. The data suggest that PGE_2 plays a protective role in 2, 3, 5-(triG-Syl)HQ-mediated cytotoxicity *via* a PGE_2 receptor coupled to the PKC signal transduction pathway. (GM 39338).

1243 EFFECTS OF HYPERTHYROIDISM ON ANTIOXIDANT SYSTEMS IN THE AGED FEMALE RAT HEART

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Hyperthyroidism (HYP) increases oxidative metabolism with profound effects on cardiac function. Both aging and HYP are associated with elevations in oxidized glutathione (GSSG), and alterations in activities of antioxidant enzymes. Since HYP occurs primarily in females, our objective was to examine the combined effects of aging and hyperthyroidism on antioxidant defenses in hearts of female rats. Young (3 mo) and aged (22 mo) Fischer 344 rats were given 0.0012% L-thyroxine (HYP) or vehicle (EUT) in drinking water for 30 days. Total glutathione (GSH), GSSG, and activities of superoxide dismutase (SOD), GSH peroxidase (GPX), GSH reductase (GRD), and catalase (CAT) were measured in heart mitochondria and S-9 fractions. Aging decreased S-9 SOD activity (50%), and increased both mitochondrial GSSG and GPX activity (50% and 40%, respectively). HYP effects on hearts of 3 mo rats were minimal: a 20% increase in heart/body weight ratio, and 30% decrease in S-9 GPX activity. In 22 mo rats, however, HYP caused a 10% drop in body weight, and 20–35% increases in heart/, liver/ and kidney/body weight ratios. HYP in 22 mo rats increased and decreased, respectively, S-9 SOD and GPX activities by 20%. One unexpected finding was that HYP in 22 mo rats decreased mitochondrial GSSG and GPX activity to levels found in hearts of young rats. These results indicate that heart antioxidant defenses are modulated to a greater extent by hyperthyroidism in aged than young female rats. Supported by Am. Heart Assn. (Texas) 94G-627.

1244 LOW-DOSE CYANIDE-INDUCED OXIDATIVE STRESS IN DUCK HEART AND BRAIN TISSUES

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Improved mining techniques over the past 10 years, which use in excess of 100 million lbs of cyanide in the U.S. yearly, have revitalized the gold mining industry. The waste from these processes results in large cyanide-containing ponds which often attract migratory birds. Migratory birds in the U.S. and other North American countries are protected by the Migratory Bird Treaty Act. These studies were designed to assess oxidative damage induced through acute exposure to cyanide-containing water in mallard ducks at concentrations below those generally accepted by the wildlife regulatory agencies as "safe" for migratory birds (50ppm). Potassium cyanide, dissolved in water, was administered to female Mallard ducks by gavage at non-toxic dosages, 0, 1 and 2mg/kg body wt. 2 hours post CN administration the animals were sacrificed and heart, liver and brain tissues excised. Superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase enzyme activities were measured in these tissues. Tissue lipid peroxidation was determined by the TBA assay. Tissue glutathione level was analyzed by HPLC. The results of these studies show that lipid peroxidation was increased in all the tissues except liver. The glutathione level was decreased in all three tissues. Catalase activity was decreased in brain and heart. The results of these studies suggest that exposure to nontoxic levels of cyanide through drinking water can result in oxidative stress. These studies were funded in part by NIEHS grant # 1 P42 ESO5961.

1245 PHENOL INDUCES MASSIVE OXIDATIVE STRESS IN MYELOTIC HL60 CELL LINE

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HL60 myelotic cells contain high levels of myeloperoxidase which catalyzes oxidation of a variety of phenolic compounds in the presence of H_2O_2. The oxidation proceeds via intermediate formation of phenoxyl radicals. Phenoxyl radicals can oxidize low M(r) reductants, *e.g.* ascorbate and GSH, and reactive protein sulfhydryls. In physiologically relevant chemical models, oxidation of thiols by redox-cycling of phenoxyl radicals was shown to release reactive oxygen species (superoxide and H_2O_2) and to hydroxylate dG in the presence of transition metals. We now report that exposure of HL60 cells to phenol and H_2O_2 resulted in oxidative stress as evidenced by: (i) depletion of GSH, (ii) oxidation of protein sulfhydryls, (iii) hydroxylation of dG in DNA, and (iv) peroxidation of *cis*-parinarioyl-residues in membrane phospholipids (phosphatitylinositol, cardiolipin, phosphatidylserine) in cells labeled with the fluorescent fatty acid probe. While H_2O_2 alone also caused oxidative stress, addition of phenol strongly increased oxidative damage. A spin trap, DMPO, significantly protected DNA and lipids in HL60 cells against phenol/H_2O_2-induced oxidative damage. DMPO did not prevent disappearance of protein sulfhydryls and GSH. Moreover, accumulation of an EPR-silent adduct of the spin trap with the glutathionyl radicals was detected in HL60 cells exposed to phenol/H_2O_2 in the presence of DMPO. The myeloperoxidase-catalyzed redox-cycling of phenol induced massive oxidative damage to HL60 cells which may be closely related to toxicity and leukemogenic effects of phenolic compounds and phenolic metabolites (*e.g.* of benzene) in myelotic cells.

1246 QUANTITATION OF FREE RADICALS IN B6C3F1 MOUSE LIVER SLICES ON EXPOSURE TO FOUR CHEMICAL CARCINOGENS: AN EPR/SPIN TRAPPING STUDY

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Free radicals generated in mouse liver slices following exposure to four chemical carcinogens: tert-butyl hydroxide (TBOOH), bromotrichloromethane ($BrCCl_3$), carbon tetrachloride (CCl_4) and trichloroethylene (TCE), were quantitated using electron paramagnetic resonance spectroscopy (EPR)/spin trapping techniques. Precision cut liver slices (n = 128) were prepared from B6C3F1 mice and incubated in Waymouth's media supplemented with 10% fetal bovine serum and 10 mM N-tert-butyl-α-phenyl nitrone (PBN). Liver slices were exposed to control media or media with 1 mM TBOOH, $BrCCl_3$, CCl_4 or TCE. After 5 or 60 min the slices and media were homogenized, frozen in liquid nitrogen and lyophilized. Radicals trapped by PBN were quantitated using an EMS 104 electron paramagnetic analyzer (Bruker Instruments, MA). Standards were prepared by homogenizing liver slices with known quantities of the spin label 2,2,5,5,-tetramethyl-1-pyrolidinyl oxy-3-carboxamide (3-CAR). EPR spectra were compared quantitatively and qualitatively. Two factorial analysis and analysis of variance (Design Ease™) showed there were significant differences in the radicals detected with time and chemical treatment ($P<0.002$). The EPR spectra of the lyophilized liver homogenate suggest there is a difference in the radical species trapped if slices are incubated in media containing 1 mM TBOOH, $BrCCl_3$, CCl_4, or TCE, when compared to control media. The interaction of both time and concentration should be addressed in any studies designed to quantitate free radical-induced liver damage.

1247 EXPERIMENTAL PARAMETERS TO SUPPORT A PHARMACODYNAMIC MODEL FOR ETHANE EXHALATION

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A computerized, physiologically based pharmacodynamic (PBPD) model was developed to simulate the kinetics of ethane generation and disposition in experimental animals. Ethane exhalation is recognized as a reliable index of lipid peroxidation which may be stimulated by prooxidant chemicals. The PBPD model was written in Continuous Simulation Language (ACSL), and simulations were performed using SIMUSOLV software with optimization capabilities (DOW Chemical Co., Midland, MI) on a VAX/VMS mainframe computer. The PBPD model simulated formation of ethane over time, depending on the concentration of the prooxidant chemical inducer, and predicted the disposition and exhalation of ethane. The partition coefficients for ethane were determined in mouse tissues *in vitro* and ethane metabolism parameters were measured with mice in a closed gas uptake chamber *in vivo*. The PBPD model allowed us to simulate the kinetics of ethane exhalation in mice treated in a preliminary experiments with massive doses of either CCl_4 or TCE. It is suggested that the PBPD model, when calibrated with experimental data for several prooxidant chemicals, may be used for future pharmacodynamic descriptions of oxidative stress and dose-response characterization of prooxidant chemicals.

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1248 ALUMINUM POTENTIATES IRON-INDUCED INTRACELLULAR OXIDATIVE STRESS IN CULTURED RAT HIPPOCAMPAL NEURONS

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Aluminum can facilitate Fe-mediated lipid peroxidation, which may contribute to Al neurotoxicity. It has been reported that Al potentiates Fe-induced intracellular oxidative stress in cultured granule cells, indicating a mechanism for Al facilitation of Fe-mediated oxidative injury. However, the relation of intracellular Al concentration to Fe-induced oxidative stress in hippocampal neurons has not been evaluated. In the present study, embryo rat hippocampal neuron cultures were treated with $Al_2(SO_4)_3$ and/or $FeSO_4$. An ionophore, A23187, was utilized to facilitate cellular Al uptake. Intracellular Al concentration was ascertained by laser microprobe mass analysis. Reactive oxygen species generated intracellularly were measured by a confocal laser scanning microscope, using 2,7-dichlorofluorescin diacetate as a probe. The study showed that increase of intracellular Al did not induce but potentiated Fe-induced intracellular oxidative stress of cultured rat hippocampal neurons. Supported by NIH ES 4640 and 7266; and the University of Kentucky Medical Center Research Fund.

1249 ENHANCEMENT OF EMBRYONIC SUPEROXIDE DISMUTASE (SOD) AND INHIBITION OF BENZO[A]PYRENE EMBRYOPATHY IN EMBRYO CULTURE BY EXOGENOUS ADDITION OF SOD

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The antioxidative enzyme SOD scavenges superoxide anions, which can be produced by peroxidase-catalysed bioactivation of xenobiotics. Since embryonic SOD activity is low, developing embryos may be at increased risk for teratogen-initiated oxidative damage. Teratogenicity of the environmental and tobacco carcinogen benzo[a]pyrene (B[a]P) may be mediated by embryonic peroxidase-catalysed bioactivation involving a free radical that initiates oxidative damage. This embryo culture study evaluated the embryotoxicity of 10 μM B[a]P compared to vehicle (DMSO) controls, and whether the addition of exogenous SOD (500 μg/ml, 1680 units/ml) could increase embryonic enzyme activity and inhibit B[a]P embryopathy. Embryos were explanted from pregnant CD-1 murine dams on gestational day 9.5 (vaginal plug = day 1) and incubated for 24 hr at 37°C in culture medium (35% male rat serum, 15% fetal bovine serum and 50% Weymouth's medium) saturated with 5% CO_2 in air. B[a]P initiated substantial reductions in anterior neuropore closure, turning, yolk sac diameter and somite development ($p<0.05$). Addition of SOD to the culture medium substantially increased embryonic SOD activity ($p<0.05$), and completely inhibited B[a]P embryopathy. These results show a strong embryoprotective role for SOD and suggest that the embryopathic effects of B[a]P occur via ROS production and oxidation of molecular targets. (Supported by the Medical Research Council of Canada)

1250 AGING AND HYPERTHYROIDISM MODULATE ACTIVITIES OF HEPATICANTIOXIDANTENZYMESIN FEMALE RATS

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BACKGROUND: One theory for aging invokes increases in reactive oxygen metabolites coupled to changes in antioxidant enzymes. Hyperthyroidism could be an additive factor since hyperthyroidism increases oxidative metabolism. **PURPOSE:** Compare effects of aging and hyperthyroidism on hepatic cytosol antioxidant enzymes including superoxide dismutase (SOD), catalase, aldehyde dehydrogenase (AIDH) and glutathione peroxidase, transferase and reductase (GPX, GST, and GSSG R). **EXPERIMENTAL DESIGN:** Young (3 mo) and aged (22 mo) Fischer 344 rats were made hyperthyroid (HYP) by giving 0.0012% L-thyroxine in drinking water for 30 days or kept euthyroid by giving just the vehicle in the drinking water. **RESULTS:** Aging decreased SOD by 68% and AIDH by 30% while increasing CAT by 89% and GPX by 44%. HYP decreased GST in young rats by 30% and in aged rats by 43%. HYP appreciably increased SOD and AIDH only in aged rats (43% and 56%). HYP strikingly increased CAT by 188% in young (188%) and to just 56% in aged rats. GPX and GSSG R were increased about 30% in the young and about 50% in the aged rats. **CONCLUSION:** This investigation indicates that hyperthyroidism modulates most hepatic antioxidant enzymes to a greater extent in aged that young female rats. Supported by AHA (TX) grant 94G-62796.

1251 *IN VITRO/IN VIVO* CORRELATION OF PARAQUAT INDUCED DEVELOPMENTAL NEUROTOXICITY

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Cells from the midbrain micromass cell culture system from rat day 13 embryos were used to investigate the developmental neurotoxicity of paraquat. Cytotoxicity and differentiation IC_{50}s were calculated. Vitamin E, a known scavenger, was added to some cultures. Lipid peroxidation was measured using the TBA assay. To correlate this with **in vivo** toxicity, dams were dosed with paraquat for 5 days (days 8–12 of gestation). On day 13 the embryonic midbrains were cultured in the micromass cell culture system. After 5 days the cultures were fixed and stained for differentiation. In an additional study, Vitamin E was administered to some dams one day prior to paraquat administration (day 12 of gestation) and then lipid peroxidation was measured by the TBA assay in the whole embryos (day 13). In the **in vitro** study there was an approximately 10-fold decrease in the IC_{50}s for differentiation (5.19 ug/mL) as compared to cytotoxicity (51.5 ug/mL). Paraquat induced a dose-related increase in lipid peroxidation as measured by the TBA assay. Vitamin E prevented the cytotoxic effects of paraquat and lipid peroxidation. In the **in vivo study** there was a significant decrease in the number of differentiating foci in the cells cultured from embryos extracted from dams dosed with paraquat. In the additional **in vivo** study, when Vitamin E was administered to dams one day prior to paraquat administration, lipid peroxidation was prevented.

1252 COPPER-CATALYZED OXIDATION OF LOW DENSITY LIPOPROTEINS (LDL) SELECTIVELY MODIFIES APO B-100 AT SPECIFIC SITES

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The oxidation of the lipid components of LDL has been studied extensively, but much less is known about the oxidation of the apoprotein. The modification of the apoprotein is likely to be a major determinant of the effects of oxidation of LDL on its function and on the process of atherogenesis. We reacted native and Cu-oxidized LDL with 2,4-dinitropheynylhydrazine (DNPH), delipidated and trypsinized the protein and analyzed the products by HPLC. At 220 nm both native and Cu-oxidized LDL showed similar patterns of protein fragmentation in their tryptic digests. However, at 365 nm, which is characteristic of DNPH-derived hydrazones, the chromatograms of oxidized LDL showed several discrete peaks, whereas native LDL showed none. Oxidations of different samples of LDL have shown qualitative similarities, with some quantitative differences. We have isolated and obtained initial structural

characterizations of selected prominent peaks from the 365 nm detection. One peptide, LEGTTR, located between amino acids 3354 and 3359, belongs to the LDL receptor binding domain, which contains a cluster of basic amino acids, such as would favor binding of a redox-active transition metal for catalysis and site direction of the oxidation. This modification of LEGTTR is one of two HPLC peaks observed after only 2 h exposure to Cu-catalyzed oxidation. Our studies suggest a surprisingly selective process of oxidation of the apoprotein and describe an approach that should be useful for the characterization of the oxidation of other proteins as well.

1253 REACTIVE OXYGEN GENERATION IN THE COCHLEA DISRUPTS COMPOUND ACTION POTENTIAL THRESHOLDS

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Reactive oxygen species (ROS) generation is believed to contribute to many forms of ototoxicity. However, the direct effects of ROS generation within the cochlea have never been studied. This study examined the functional effects of directly infusing H_2O_2 or generating systems for the superoxide anion (O_2) or the hydroxyl radical (OH.) into the cochlea. Anesthetized guinea pigs received surgery to open the auditory bulla. A round window electrode was used to record compound action potential (CAP) thresholds and 1.0 μV RMS cochlear microphonic (CM) isopotential values for ten pure tone acoustic frequencies (2–35 kHz). A fenestra was then produced in the otic capsule, and potentials were again recorded. H_2O_2 (1.0 mM) or a generating system for O_2^- (xanthine-xanthine oxidase) or OH. (xanthine-xanthine oxidase plus $FeCl_3$) in artificial perilymph (AP) was then instilled into the scala tympani. Other groups received AP control infusions, or specific ROS scavenging agents in addition to their respective ROS generating system (50,000 U/l superoxide dismutase for O_2^-, 10,000 U/l catalase for H_2O_2 or 1.0 mM deferoxamine for OH.). Mean CM and CAP values remained stable over the 120 min post-exposure period in the control group. In each group receiving an ROS generating system, mean CAP threshold shift values were significantly elevated relative to control values at all mid-high frequencies ($p < .05$); mean CM values were not significantly different than control values. Each group receiving a scavenging agent plus an ROS generating system had significantly ($p < .05$) reduced mean CAP threshold shifts relative to those receiving the respective ROS generating system alone. These data suggest that ototoxic agents which promote cochlear ROS generation may preferentially degrade mid-high frequency CAP function.

1254 PEROXIDETOXICITY AND BCL-2 IN A549 CELLS

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The protooncogene *bcl-2* was discovered through studies of the t(14; 18) translocation present in most B cell lymphomas that results in increased production of the bcl-2 protein. This enhances cell survival in the presence of many agents including oxidants and free radicals. The physiological function of the 26 kDa *bcl-2* encoded protein is not known but a link between free radicals, apoptosis and *bcl-2* is supported by several studies. The present study assessed the effect of acute *t*-butyl hydroperoxide (tBOOH) exposure on the expression of bcl-2 protein in human lung carcinoma A549 cells. Basal bcl-2 protein levels, as assessed by western blots with ECL detection, were low in A549 cells. Exposure of A549 cells to 1, 5, or 10 mM tBOOH for 4 h resulted in the respective loss of 61, 73, and 87% of a protein that had mobility characteristics similar to bcl-2. This loss did not appear to be due to a general decline in protein. Although phase contrast microscopy showed only a modest decrease in cell number over the 4 h exposure period, there was a large decrease in cell viability with all doses of tBOOH as reflected by LDH leakage and trypan blue uptake. Condensation of nuclear material was evident and Coulter counter analyses showed a decrease in the volume of cells exposed to 5 or 10 mM tBOOH. Trypsinization prior to cell size analyses lysed many cells, particularly at 1 mM tBOOH, presumably due to an increase in membrane fragility. These data suggest that acute oxidant injury may deplete bcl-2 protein contents. Both apoptosis and necrosis may be occurring at these doses of peroxide. Additional dose/response and time course studies, and studies with other toxicants, are needed to assess the possible relationship between loss of bcl-2 protein and acute oxidant injury or apoptosis. This work was supported by grant HL 51005 (JPK) and GM44263 (CVS).

1255 INCREASED FERRITIN HEAVY AND LIGHT CHAIN HEPATIC mRNA LEVELS FOLLOWING DIQUAT ADMINISTRATION IN FISCHER 344 RATS

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Diquat (DQ) is a bipyridyl herbicide that induces hepatotoxicity in vivo, secondary to oxidative stress. Ferritin is a ubiquitous iron storage protein, the subunits (H) and (L) of which are differentially regulated, resulting in a mixture of tissue- and condition-specific isoforms. In these studies, we examined the effects of DQ on hepatic ferritin mRNA levels *in vivo*. DQ induced hepatotoxicity, as indicated by clinical chemistries and histopathological assessment (necrosis and inflammatory cell infiltration). Pretreatment with U-74500A or desferrioxamine inhibited all indicators of hepatotoxicity. DQ-dependent increases in L-chain mRNA were detectable at 1.5 hours and maximal at 12 hours (4.8-fold). H-chain mRNA increases were not detectable before 3 hours, but thereafter continued to increase through 24 hours (9.6-fold), the latest time-point measured. The L-chain mRNA increases were similar to those observed in iron overload, consistent with the hypothesis that DQ hepatotoxicity is at least partially iron-mediated. The timecourse of the H-chain mRNA increases were also consistent with literature reports that H-chain mRNA is inducible by cytokines, as inflammatory cell infiltration followed a similar timecourse. However, while antioxidants were able to block hepatotoxicity/inflammatory cell infiltration, DQ-dependent increases in ferritin mRNA levels were not similarly inhibited. Additional studies are necessary to further elucidate the mechanism(s) by which DQ affects ferritin gene expression.

1256 IN VITRO FREE RADICAL PRODUCTION IN HUMAN KERATINOCYTES INDUCED BY SMOKELESS TOBACCO EXTRACT (STE)

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Smokeless tobacco increases oral cancer risk and other cancers. However, the mechanism by which smokeless tobacco constituents produce these effects is not clear. Cells were obtained from the oral cavities of human subjects and grown to 100% confluency in keratinocyte growth medium (KGM), supplemented with pituitary extract). Approximately 70% confluent primary normal human oral keratinocytes (NHOK) grown in 60 mm petri dishes were subcultured successfully in 6-well plates, treated with N50-300μg/ml concentrations of an aqueous extract of smokeless tobacco (STE) and incubated for 24 hours at 37°C. The overall intracellular oxidized states of cells were measured by laser scanning confocal microscopy (MRC 500: Biorad Laboratories, Richmond, Ca). 2, 7-Dichlorofluorescein diacetate (DCFH) was used as the fluorescent probe (Molecular Probes, Inc., Eugene, OR). The excitation wavelength of 513 nm was used. The intensity of DCFH fluorescence increased with the increasing concentrations of STE. Intensities were approximately 5–7 fold higher in STE treated cells as compared to untreated cells. Some cells showed high fluorescence in nuclei, possibly because of accumulation of the dye in nuclear fractions. These results suggest that STE treatment leads to an increase in intracellular oxidation states of keratinocytes. Protein kinase C (PKC) may be a target of ROS and lead to altered cell proliferation. Therefore, NHOK's were exposed to STE(50–300 μg/ml) for 3 to 24 hours at 37°C, homogenized and PKC activity was assessed. STE significantly increased PKC activity in cells. The total PKC activity in cells exposed to STE was about 1.5–2.0 times that of control cells after 3 and 24 hrs of incubation. Keratinocytes were further treated with (5–50μg/ml) of STE for 24 hrs. RNA was isolated from treated and untreated cells. Plasmid (Stratagene, CA) containing cDNA encoding the NCI domain of L_3, L_4, and L_5 chains of Type IV collagen were isolated. The L_3, L_4, and L_5 cDNA were used as probes to detect respective Type IV collagen mRNA from treated and untreated NHEK cells. The data indicate that exposure of human keratinocytes to aqueous extracts of STE can result in the production of highly reactive forms of oxygen (ROS) which can adversely affect biosynthetic pathways of collagen synthesis.

1257 PROTECTIVE EFFECT OF NITRIC OXIDE SYNTHASE (NOS) INHIBITORS AGAINST QUINOLINIC ACID (QUIN) NEUROTOXICITY

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QUIN is a potent excitotoxin acting on NMDA receptors. Two end-points of QUIN neurotoxicity are circling behavior (CB) and lipid peroxidation (LP). It has been proposed a role of nitric oxide as a mediator of QUIN injury. Both **In vivo** and **in vitro** QUIN toxicity was tested in the presence of inhibitors of NOS activity, such as L-nitro arginine methyl ester (L-NAME). QUIN-induced CB (observed after apomorphine challenge) and LP (evaluated by lipid fluorescent products) were measured in striatally-lesioned rats and brain synaptosomes, respectively. QUIN (240 nmol/μl) - mediated CB (265 turns/hr) was prevented by 5, 10 and 15 mg/kg of i.p. L-NAME (-25, -49 and -52 %, respectively, as compared vs QUIN-treated rats). QUIN (10 mM) -induced LP was 156 % vs control and L-NAME (10, 20 and 40 mM) prevented LP by -52, -42 and -51 % vs QUIN, respectively. Similar results were observed with other NOS inhibitors. These findings suggest a contribution of NOS activity to the QUIN-induced brain injury (CONACYT grant 3525-M).

1258 CHARACTERIZATION OF POLYCLONAL ANTIBODY AGAINST 8-EPI PROSTAGLANDIN $F_{2\alpha}$ (8EPGF2)

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A sensitive and specific immunoassay for 8EPGF2 has been developed to facilitate investigations of the physiologic and pathophysiologic roles of the compound, and clinical assessment of oxidative status. Anti-8EPGF2 IgG did not cross-react with enzymatically produced prostaglandins, and 10-1000 pg of 8EPGF2 can be measured in ELISA (Kim et al., The 9th International Conference on Prostaglandins & Related Compounds, 1994). The levels of 8EPGF2 in urine assayed prior to purification of 8EPGF2 using C18 column chromatography were higher than those obtained after the treatment, whereas 100% of radioactivity of ^{3}H-8EPGF2 applied to the column was recovered after the same treatment. These results show that the antibody against 8EPGF2 cross-reacts with both free 8EPGF2 and unidentified 8EPGF2-related substances in urine. The levels of 8EPGF2 of oxidized low-density lipoprotein (LDL) were higher than those of LDL, when extracted lipids from the lipoproteins were subjected to ELISA with or without treatment with yeast lipase. These results suggest that the levels of 8EPGF2 increase during LDL oxidation, and anti-8PGF2 IgG recognizes both free and esterified 8PGF2. Supported by NIH grant GM47634.

1259 INDUCTION BY 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) OF PROSTAGLANDIN H SYNTHASE (PHS) AND PEROXIDASE-DEPENDENT, BENZO[A]PYRENE (B[A]P)-INITIATED PROTEIN OXIDATION IN THE MECHANISM OF B[A]P GENOTOXICITY

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B[A]P is bioactivated by cytochrome P4501A1 (CYP1A1) and PHS to reactive intermediates that initiate reactive oxygen species, DNA oxidation and micronucleus formation in Wistar rat skin fibroblasts. In this study, cultured Wistar rat skin fibroblasts were preincubated with the dual peroxidase/CYP1A1 inhibitor 1-aminobenzotriazole (1 mM ABT), or PHS inducers 0.0367 nM interleukin-1α (IL-1α) or 1μM 12-O-tetradecanoylphorbol-13-acetate (TPA), prior to adding 10 μM B[a]P. Protein oxidation was measured via Western blot/immunodetection. PHS and CYP1A1 protein induction was measured similarly in cells incubated with either the CYP1A1 inducer 10 nM TCDD alone, B[a]P alone, or ABT plus B[a]P. B[a]P alone substantially increased protein oxidation over TPA or IL-1α alone or untreated controls, which had no effect. B[a]P protein oxidation was blocked by ABT and increased by IL-1α or TPA. Either B[a]P or TCDD alone induced CYP1A1 protein, which was blocked by ABT. Constitutive PHS protein was evident in untreated cells and was induced by TCDD, but not by B[a]P. This study implicates protein oxidation as an additional component of previously reported B[a]P-initiated oxidative stress as a potential mechanism in micronucleus formation, and suggests that PHS induction may be important in TCDD-enhanced toxicities. (Support: Medical Research Council of Canada)

1260 BIOACTIVATION OF PHENYTOIN, THALIDOMIDE AND RELATED TERATOGENS TO A FREE RADICAL INTERMEDIATE USING PROSTAGLANDIN H SYNTHASE (PHS) OR HEPATIC MICROSOMES: CHARACTERISATION BY ELECTRON SPIN RESONANCE (ESR) SPECTROMETRY

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Embryonic PHS-catalysed bioactivation of phenytoin and related teratogens (mephenytoin, nirvanol, trimethadione, dimethadione and thalidomide) to a reactive free radical intermediate may initiate teratogenesis. PHS (1000 U/ml) was incubated with hematin (1.0 μM) and phenol (0.5 mM) for 1 min at 37°C in an 80 mM potassium phosphate buffer (PBS), pH 7.9. After addition of the teratogen (500 μM) or its vehicle and the free radical spin trap α-phenyl-N-t-butylnitrone (PBN, 1 mM), arachidonic acid (67 μM) was added to start the reaction. After 30 min at 37°C, reactions were stopped and extracted twice with 2 ml ethylacetate. The combined ethylacetate layers were reduced under nitrogen to 500 μl and analysed by ESR spectrometry. There was no radical adduct of PBN in control incubations. For phenytoin, a mixture of two carbon-centred radical adducts were observed as a triplet of doublets. The hyperfine splitting constants (hfscs) of the principle free radical adduct were $a^N = 13.73$, $a\beta^H = 2.45$; and $a^N = 12.92$, $a\beta = 3.73$ for the second adduct. Free radical adducts also were detected in varying amounts for all the other teratogens, and all except thalidomide exhibited hfscs similar to that for the principle free radical adduct of phenytoin. Addition of the PHS inhibitor of eicosatetraynoic acid (40 μM) abolished the free radical ESR signal. Incubating phenytoin with CD-1 mouse hepatic microsomes (3 mg/ml) and PBN in PBS (0.1 mM, pH 7.4) in a total volume of 3 ml for 60 min at 37°C produced the same principle radical-PBN adduct observed with PHS. These results provide the first definitive evidence for PHS-catalysed bioactivation of phenyoin and related teratogens to a free radical intermediate, which may constitute a common molecular mechanism of teratologic initiation. (Supported by Medical Research Council of Canada)

1261 PHOTOLYTIC OXIDATION OF PHENYTOIN USING SODIUM PERSULFATE TO MODEL PHENYTOIN BIOACTIVATION BY PROSTAGLANDIN H SYNTHASE (PHS) TO A FREE RADICAL INTERMEDIATE

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Teratogenicity and idiosyncratic adverse reactions of phenytoin may result from PHS-catalysed bioactivation to a free radical intermediate that oxidises or covalently binds to cellular macromolecules. To determine the chemical mechanism of formation of this free radical, photolytic oxidation with sodium persulfate was used to model PHS-catalysed bioactivation of phenytoin. A 3 ml solution containing 100 μM phenytoin and 100 μM sodium persulfate in 6.0 mM NaOH (pH 11.8) was photolysed at 300 nm for up to 40 min in a Rayonet chamber, and the products were detected by high-performance liquid chromatography (HPLC) with UV detection. Over 50% of phenytoin was photolysed to one major and 3 minor products, which were tentatively identified by HPLC-tandem mass spectrometry to be: (1) 1,2,3,4-tetrahydro-2-oxo-4-phenylquinazoline, (2) 1,1-diphenylmethylhydroxy imine, (3) benzophenone, and (4) 1-phenyl-1-[2-hydroxyphenyl]methyl imine. These preliminary studies suggest that phenytoin is first photolysed to an unstable nitrogen-centred radical that rapidly undergoes ring opening to form a carbon-centred radical. This carbon-centred radical could form a carbocation through one-electron oxidation by persulfate leading to formation of the above stable compounds. These studies are consistent with the similar in vitro formation of a carbon-centred radical of N-bromosuccinamide, an analogue of phenytoin. These results provide a chemical basis for the observed PHS-catalysed bioactivation of phenytoin to a carbon-centred free radical intermediate (Parman et al., 1996, accompanying abstract), which may initiate teratogenesis and adverse drug reactions. (Supported by the Medical Research Council of Canada)

1262 THE ROLE OF INTRACELLULAR CALCIUM OVERLOAD AND OXIDATIVE STRESS IN MECHANISMS OF NEUROTOXICITY

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Endogenous intoxication of CNS is developed as a result of poliorgan pathology, is a consequence of hard lesion of main organs of detoxication of liver and kidney. The aim: the investigation of some mechanisms of development of neurotoxicity at endotoxemia of hepatorenal ethiology. 210 patient with terminal stage of chronical liver (I group) and kidney (II group) failure, complicated by the development of metabolic encephalopathy were examined. Neurostatus of patients, the status of higher grey matter functions, electrolite exchange, the processes of LOP and antioxidant defence were studied. The investigations showed, that the patients of two groups have the disseminate organic neurological simptomatics in the form of central pares VII-XII pairs of cranial nerves, piramidal simptomatics, pathological reflexes, cerebellum simptomatics. The violation of status of high grey matter functions-short-term memory, attention, the time of complex moving reaction, the tremor of hands had 77, 0+7, 6% of I group patients and 76, 0+7, 5% of II group patients. The analyse of Ca++ content in serum blood showed the expressed reducing of it in 100% patients of the I group and in 90, 9% of the II group of patient. The status of oxidation stress with the increasing of AHP and MDA content in serum blood was revealed. The reducing of intracell scavengers—SOD and catalase content in two groups of patients was observed. The correlation between the reducing of Ca++ content in serum blood and the increasing of MDA content in serum blood for the I group (r=0,85) and for the II group of patients (r=0,82) was revealed. The correlation between the reducing of Ca++ content in serum blood and the reducing of parameters of short-term memory for the I group (r=0,87) and for the II group of patients (r=0,92) also was revealed. Therefore, the excessive entering of Ca++ ions in cerebral cells which leads to the induction of neurodegeration processes and its apoptosis are being observed in the condition of generalization of LOP processes.

1263 A PHYSIOLOGICALLY-BASED KINETIC MODEL FOR DIETARY UPTAKE OF HYDROPHOBIC ORGANIC COMPOUNDS BY FISH

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A physiologically-based toxicokinetic (PB-TK) model was developed to describe dietary uptake of hydrophobic organic compounds by fish. The gastrointestinal tract was represented by three compartments corresponding to the stomach, upper and lower intestine. Gastric emptying time, volume reduction of food, and assimilation of lipid, protein and organic carbon were determined for 100 g rainbow trout fed 60 d-old fathead minnows. The minnows were then exposed to 2,2'5,5'-tetrachlorobiphenyl (TCB) to provide contaminated prey for subsequent kinetic studies. Diffusion parameters for each gut segment were fitted by modeling to blood, tissue and lumenal TCB residues. Based upon this analysis it appeared that diffusion and/or desorption limitations retarded chemical uptake from the stomach. Instead, chemical flux followed lipid assimilation in the upper intestine. This occured when much of the ingested meal (dry wt) was still in the stomach, suggesting the importance of liquid-phase emptying. Feces retained an appreciable affinity for TCB despite nearly complete uptake of lipid. This may account for the relatively low (50–60%) net assimilation of TCB and related compounds in feeding studies with fish, although branchial elimination is predicted to contribute substantially when chemical log P is < 7.0.

1264 SIMULATION OF TOLUENE KINETICS IN THE RAT USING BIOTRANSFORMATION PARAMETERS DERIVED *IN VITRO*

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The biokinetic behaviour of toluene was modeled in the rat with a physiologically based pharmacokinetic (PB-PK) model. The model was parameterized by using reference physiological parameter values and partition coefficients that were reported earlier from *in vitro* studies. Biotransformation parameters for toluene, reported from two *in vivo* and six *in vitro* studies, were subsequently substituted in the model while keeping all other model parameters constant. Simulations of toluene kinetics, based on these eight biotransformation parameter sets, were compared with empirical data reported on toluene uptake in blood and/or brain tissue after inhalation exposure. It was observed that the empirical data on toluene blood concentrations were adequately predicted by the two *in vivo* derived biotransformation parameter sets and by five of the six *in vitro* derived biotransformation parameter sets. It was also observed that differences between model predictions, based on either *in vivo* or *in vitro* derived biotransformation parameters, were small for most of the simulated exposure scenario's. It is concluded that the results from most *in vitro* studies on toluene biotransformation can be applied to successfully predict the kinetics of toluene *in vivo*. Based on sensitivity analysis of the model, it is also concluded that physiological parameters and partition coefficients may be at least as important for the outcome of the model as the biotransformation parameters are. These results support earlier reported findings in the literature on the application of *in vitro* techniques to derive parameters for PB-PK models.

1265 SENSITIVITY ANALYSES OF INPUT PARAMETER EFFECTS ON INTERNAL DOSIMETRY IN PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELING OF CARBON TETRACHLORIDE (CCl_4) AND TRICHLOROETHYLENE (TCE)

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With current interest in PBPK modeling as a tool to predict target dose of environmental toxicants, an understanding of the relationship between physiological parameters and model output is necessary to increase confidence in models. This study was conducted to evaluate these relationships in rats exposed to CCl_4 and TCE using sensitivity analyses. Input physiological parameters used in model simulations were either EPA reference values or values measured in male S-D rats in our laboratory for cardiac output, tissue volumes and blood flows. Previous simulations have shown significant differences in fat or liver concentrations and amount metabolized for both compounds when the two different sets of physiological inputs were compared. Normalized sensitivity coefficients (SCs) were calculated for 6 hr, 1 and 1,000 ppm inhalation exposures to CCl_4 (slowly metabolized) and TCE (rapidly metabolized). In general, SCs for TCE were numerically higher than those for CCl_4, indicating that physiological parameter inputs are more critical for TCE. For example, when fat and liver concentrations or amount metabolized were evaluated for TCE, the physiological inputs having the largest effect on model estimates were ventilation rate, cardiac output and % liver flow. The results illustrate how sensitivity analyses may be used to determine where efforts should be devoted to improve model inputs, to facilitate validation and increase information. (This abstract does not necessarily reflect U.S. EPA policy. Supported by EPA Cooperative Agreement CR-820447).

1266 PHARMACOKINETICS AND PHARMACODYNAMICS OF 3,3',4,4'-TETRACHLOROBIPHENYL IN THE RATS: PBPK MODEL DEVELOPMENT

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Lipophilic and stable polychlorinated biphenyls are ubiquitous environmental contaminants and exposure of PCB77 to the rats induces CYP1A1 enzyme activity. The objectives of this study were (1) to investigate the disposition and hepatic enzyme induction in the rats following a single ip injection of PCB77 and (2) to develop and validate a PBPK model. Male Fisher 344 rats (n=4) were dosed with radiolabeled PCB77 (0.5 mg and 5 mg/ kg bw) in corn oil and control rats received corn oil only. At low doses, the exposed rats were sacrificed at eleven time points till Day 16 and at high doses twelve time points till Day 44. Slow uptake and clearance in the fat were described by a diffusion-limited process while other tissues was described by a flow-limited process. The ratio of PCB77 concentrations in the liver to fat was time-dependent and PCB77 concentration was higher in the fat than liver after Day 1 post dosing. Uptake of PCB77 to the systemic system and elimination of PCB77-derived radioactivity in the feces were described as the first order process. Induction of hepatic ethoxyresorufin O-deethylase (EORD), a biomarker for CYP1A1, was dose-dependent and Michaelis-Menten-type equation was used to described EROD induction. Rats were exposed to six different concentrations of PCB77 (0.1, 0.5, 1, 5, 10, and 50 mg/ kg bw) and sacrificed them on Day 3 to validate the PCB77 model. Model estimation of kinetic behavior in the liver and fat, and EROD induction were adequately described by the model except for the 50mg/kg bw.

1267 DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR TRICHLOROETHYLENE AND ITS METABOLITES IN B6C3F1 MICE

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In recent years, three carcinogenicity bioassays have indicated that the daily oral administration of TCE in corn oil lead to an increased incidence of hepatocellular carcinoma in B6C3F1 mice. To describe and predict the kinetics of TCE absorption, blood and tissue distribution, metabolism and excretion, and better understand its toxicity, a PBPK model was developed for TCE and its metabolites in mice. This model provides a detailed description of TCE kinetics that combined with relatively simple sub-models for metabolites. The model structure consisted of liver, kidney, lung, richly perfused tissues, slowly perfused tissues and fat that were interconnected by arterial and venous blood pools. Partition coefficients (PCs) for TCE were determined by vial equilibrium method, and PCs of non-volatile metabolites were determined using the methods of Jepson et al. (1994). B6C3F1 mice were given bolus oral doses of 300, 600, 1200 and 2000 mg/kg TCE dissolved in corn oil. At various time points, mice were sacrificed and blood, liver, kidney, lung and fat were collected. The blood and tissue samples were assayed for TCE and its metabolites, chloral hydrate (CH), trichloroethanol (TCOH), trichloroethanol glucuronide (TCOG), trichloroacetic acid (TCA) and dichloroacetic acid (DCA). This study demonstrated that DCA was formed in mice and its formation and kinetics appeared to be driven by TCA concentration. (Supported by SERDP grant CU-110).

1268 A DETAILED COMPARISON OF THE VARIOUS MODELS USED TO PREDICT BLOOD LEAD CONCENTRATIONS FOR A GIVEN EXPOSURE SCENARIO

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Lead has been and will continue to be a major concern as an environmental contaminant. The major concerns with lead poisoning are neurotoxic effects. The Center for Disease Control (CDC) has issued a benchmark for a safe blood lead concentration of 10 μ/dL. Since this criteria is an internal concentration and not an exposure concentration, models are needed to predict the blood lead concentration associated with a given exposure scenario when performing a site specific risk assessment. The USEPA recommends the Integrated Exposure Uptake BioKinetic (IEUBK) model for predicting blood lead concentrations in children less than 6 years of age but has not made any official recommendations as to which model should be used for predicting blood lead concentrations in adults and/or children older than 6 years of age. Three lead models (IEUBK, a physiologically-based pharmacokinetic (PBPK) model and an empirical kinetic model (Bowers)) were compared to determine which model is most appropriate for use in site specific risk assessments. These models were compared as to; 1) model structure, 2) model parameter values and sensitivity in affecting the model predictions, 3) extent and applicability of model validation, 4) differences in model predictions for the same exposure scenarios, and 5) the theoretical limits of application. The PBPK model was found to be the most fully validated, and was the only model applicable to predict blood lead concentrations associated with short-term exposures and for both children and adults. The IEUBK model was found to contain some highly sensitive model parameters which were empirically derived and was not valid for modeling short-term exposure scenarios. The Bowers model was found to be of limited scope and complexity and was only theoretically applicable for predicting blood lead concentrations in adults exposed to lead for long durations.

1269 EXTRAPOLATION OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL DESCRIBING 2-METHOXYACETIC ACID DISPOSITION AFTER 2-METHOXYETHANOL INHALATION AT 5 PPM FROM PREGNANT MICE TO RATS AND HUMANS

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Accurate prediction of embryo dosimetry in pregnant animals is an important step towards improved risk assessment for exposure to chemicals during human pregnancy. We have developed a PBPK model that describes the disposition of 2-methoxyacetic acid (2-MAA) in mice and their embryos during organogenesis (*TAP* 132, 103-114, 1995). This alkoxyacid is the primary metabolite and proximate toxicant of 2-methoxyethanol (2-ME). The model was extrapolated to pregnant rats with pharmacokinetic data obtained from oral and iv bolus administrations of 2-ME (*Toxicologist* 15, 47A, 1995). Subsequently, the model accurately predicted maternal plasma levels of 2-MAA determined when mice inhaled 5 ppm 2-ME for 8 hr on gestation day 11. Inhalation exposures (5 ppm; 8 hr/day; 5 days/week) were simulated for rats and women during the first 3 weeks of pregnancy. Projected 2-MAA plasma levels were well below those causing grossly visible malformations in mouse fetuses. Based on animal studies, maternal plasma 2-MAA concentrations might be suitable surrogates for those in the embryo. The same model also reasonably fitted published 2-MAA urinary excretion data from men who had inhaled 5 ppm 2-ME for 4 hr. Taken together, the projections show that this PBPK gestation model provides an improved quantitative basis for extrapolations among species and from high to low dose, which are important components of the risk characterization process.

1270 APPLICATION OF NICOTINE PHARMACOKINETIC MODELING FOR DATA ANALYSIS

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Physiologically- (PBPK) and population-based nicotine pharmacokinetic / pharmacodynamic models have been applied to describe nicotine plasma kinetics and to estimate nicotine uptake in cigarette smokers. These models have also suggested optimal designs for pharmacokinetic experiments, and explained some of the physiological effects of nicotine. The selection of a model for data analysis depends upon the nature of the available data and the objective of the analysis. Current work seeks to evaluate modeling approaches for analyzing sparse data which were obtained as a supplement to another study. Both PBPK and population models described the plasma data reasonably well. Nicotine uptake from smoking a commercial cigarette was estimated in subjects by applying a validated PBPK model to plasma data. The model-estimated value of 0.95 mg/cigarette was in line with nicotine yields measured by the human mimic smoke machine (1.11 mg/cigarette) and the FTC method (0.71 mg /cigarette). Model estimates may have been impacted by interindividual differences in physiological or/and biological factors. However, the confidence in the model would be greatly strengthened if sampling times and data points were optimized. Although the interindividual variability in pharmacokinetic parameters was taken into account by a population modeling approach, uncertainty remains concerning the bioavailability of nicotine based on current data. This uncertainty results from the nature of the data and the fact that an IV reference data set is not available. Nevertheless, plasma kinetics as calculated by interpolation from a population model, previously derived from well balanced and controlled experiments, can be linked with response to nicotine to develop a pharmacodynamic model.

1271 PHARMACOKINETICS OF [^{14}C]TRICHLOROACETATE IN MALE FISHER 344 RATS

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The pharmacokinetics of trichloroacetate (TCA) are being investigated experimentally and analyzed using a physiologically based pharmacokinetic (PBPK) model. TCA is a metabolite of both trichloroethylene and perchloroethylene as well as being a chlorination byproduct in drinking water. TCA causes liver cancer in mice, but not in rats. It also causes cardiac malformation in the offspring of rats exposed during pregnancy. TCA is poorly metabolized (<20%) and largely excreted in urine. It has a pKa of 0.9; it is fully charged at physiological pH, except in the stomach. Rats were dosed with 10 mg/kg by *i.v.* injection via the lateral tail vein. Blood and tissues were collected at 0.5, 1, 3, 6, 9, 24, and 48 hr post injection (n = 4 rats/time). Total radiolabel was determined in oxidized tissues, urine, and exhaled CO_2 (trapped in aqueous potassium hydroxide) by scintillation counting.. In a separate series of experiments blood was sampled via a jugular canula to determine concentrations at early time (2, 6, 12, 18, 24, 30, 36, 46, 60 min). Radiolabel concentrations were highest in plasma until late times. Virtually all plasma radioactivity was TCA (determined by HPLC). Tissue concentrations at 0.5 hr: Plasma > Kidney > Red Blood Cells > Liver > Large Intestine > Skin > Small Intestine > Muscle > Fat. A parallel linear decrease occurred out to 9 hr in plasma, kidney, red blood cells, skin, muscle, and fat. Concentrations in liver and small and large intestine decreased more slowly. A PBPK model is being developed that includes compartments for plasma, red blood cells, liver, kidney, and other lumped tissues. The goal is to model the tissue

kinetics of TCA by including factors such as tissue water volume and protein binding in the PBPK model.

1272 IN VITRO HEPATIC METABOLISM OF PCBTF: DEVELOPMENT OF V_{MAX}, K_M VALUES AND THEIR USE IN A PBPK MODEL

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Liver microsomes were harvested from male and female rats previously exposed to 0, 10, 50 and 250 ppm PCBTF (p-chlorobenzotrifluoride) in a 90-day inhalation neurotoxicity study (Toxicologist Vol 15, abstract 444, 1995) and unexposed rats to determine total P-450 and induced enzyme activity against PCBTF as measured by changes in V_{max} and K_m values. Values were determined by incubating PCBTF (0.2, 0.4, 0.8, 1.2 and 1.6 umoles) for 60 min in 10 ml of 0.1 M phosphate buffer, pH 7.4, containing 1.3 nmoles of P-450 and 8.0 umoles of NADPH or an NADPH regeneration system in sealed 20 ml vials. At the end of the incubation period, 5.0 ml of the water phase were extracted with 2.0 ml of hexane and 2.0 ul analyzed by gas chromatography for 3-OH-PCBTF. V_{max} and K_m were determined by plotting V_o vs V_o/[S]. For comparison, the activity of a commercial AROCLOR 1254-induced S9 preparation containing 1.3 nmoles of P-450 toward PCBTF was determined under similar conditions. The V_{max}, K_m values obtained in the study were used in a rat PBPK model describing the fate of inhaled PCBTF. The model was validated using inhalation pharmacokinetic data (6 hrs of exposure) obtained using female rats previously exposed for 90-days to PCBTF.

1273 DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR METHANOL IN PREGNANT RATS AND MICE

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Physiologically based pharmacokinetic (PBPK) models have been developed in recent years to describe the disposition of toxicants during gestation. These models can account for the dynamics of physiological changes associated with pregnancy and represent a significant advantage in quantitatively assessing potential exposure of the conceptus. We have developed a PBPK gestation model for methanol (M). To validate this model, levels of M in dam and conceptus were determined after M exposure of rats on gestation days (gd) 14 and 20 and of mice on gd 9 and 18 by inhalation or oral or iv bolus. Typically, M concentrations in conceptuses exceeded those in dams by ~25%. At the developmental stages examined, the model provided a good description of M disposition in either target organism of both species. Simulated kinetic parameters were similar to those obtained by conventional compartmental models of M toxicokinetics. V_{max} for M elimination in adult animals was ~2 times greater in mice than in rats; K_m was similar for both species. V_{max} in both species decreased with length of gestation; at term, V_{max} was only ~80% of the non-pregnant value. The model demonstrates the importance for risk characterization of accurate descriptions of a chemical's disposition across gestational stage, dose levels, species, and classes of toxicants. (Supported by HEI #90–10; NIEHS T32 ES07126).

1274 THE MODELING OF DIFFUSION-LIMITED COMPOUNDS IN PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELS

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Most PBPK models to date have been for the description of the uptake, distribution, metabolism and elimination of low molecular weight compounds. These compounds, because of their relatively small size, diffuse across cellular membranes relatively rapidly. Analyzing the rates of delivery of a chemical via blood flow to an organ versus the rate of diffusion across cellular membranes and the rates of metabolism suggest that the overall rate-limiting factor for the elimination of these low molecular weight chemicals is flow. The PBPK modeling of larger chemicals such as polycyclic aromatic hydrocarbons (PAHs) adds new complexities because their fates are, in most cases, diffusion-limited (i.e., the rate of diffusion across the cellular membrane is the rate-limiting step for the overall elimination of chemical by an organ). The classical model of diffusion of chemicals across membranes and through material relates the overall rate of mass transfer to the diffusivity, surface area over which the chemical is diffusing and the concentration gradient between the two sides. The approach used in most PBPK models to describe the diffusion of chemicals across organ membranes relates the mass transfer of chemical across the organ membrane to the blood flow rate to that organ, an organ-specific mass transfer factor, and the concentration gradient. The problems associated with this type of simplification are discussed and an alternative is proposed. It is shown with PBPK model simulations that the proposed way of modeling diffusion-limited chemicals is more appropriate.

1275 DEVELOPMENT OF PBPK MODELS FOR Hg^0 AND CH_3Hg AS TOOLS IN UNDERSTANDING INORGANIC Hg DOSIMETRY IN THE BRAIN

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Human exposure to Hg species most associated with neurotoxicity are from vaporous Hg^0 exposures in occupational settings and MeHg contamination of fish that are the principle diet of some populations. Human toxicity from exposure to Hg^0 has been hypothesized to be primarily due to formation of the mercuric (Hg^{2+}) metabolite of Hg^0 in target tissues. This metabolic pathway has been shown experimentally to be the result of oxidation of Hg^0 via catalase complex I. The metabolism of MeHg in different tissue sites of the mammal results in the intracellular formation of inorganic mercury (I-Hg). While it has been shown in *in vitro* studies that MeHg is itself neurotoxic, it is not clear to what extent the formation of the MeHg metabolite, I-Hg, has on the toxicity of MeHg in the central nervous system. The goal of this project was to develop physiologically based pharmacokinetic (PBPK) models for Hg^0 and MeHg which could be validated with data available in the literature, and which could account for Hg^{2+} formation in the different tissues. A PBPK model was developed for Hg^0 inhalation assuming flow limited kinetics and using the physiological parameters for different mammalian species from the literature. The metabolism of Hg^0 to Hg^{2+} was assumed to occur in all tissues except fat, as shown from experimental animal and human studies exhibiting long term clearance of Hg from all tissues. Experimentally determined human partition coefficients from the literature for Hg^0 were used to simulate controlled human Hg^0 inhalation exposures. A previously developed PBPK model for MeHg pharmacokinetics was used to predict MeHg and I-Hg concentrations in brain tissue. A comparison of I-Hg formed in brain tissue after exposure to either Hg^0 or MeHg was made with output from the different model simulations, suggesting a dominant role for Hg^{2+}. These simulations form the basis for understanding the extent and time course of neurotoxic response after exposure to these two neurotoxicants.

1276 A NEW DISTRIBUTIVE COMPUTING APPROACH TO PB-PK MODELING REDUCES PROGRAMMING KNOWLEDGE BY TOXICOLOGISTS AND ALLOWS DISTANT INTERACTIVE PARTICIPATION BY EXPERIMENTERS

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Mathematical modeling has greatly increased the quantitative use of toxicology data, especially in estimating tissue or organ doses. Current methods require advanced knowledge of programming and are labor intensive. The methods now in use are for single investigators and do not allow multiple investigator interactions. Simulations are batch computed so that it is not possible to reverse the simulation in simulation time nor to interact with the simulation parameters. Using autonomous objects, we have demonstrated that a styrene inhalation PB-PK model can be implemented which provides the same precision and sensitivity as the original. In our system each organ is a node receiving intelligent instructions, which we call MESSENGERS, from the originating node.

MESSENGERS support mathematical descriptions of all toxicological phenomena. The tools are accessed by a graphics interface, freeing toxicologists from the repetitive labor of conventional programming. Using MESSENGERS interaction and cooperation between geographically separated toxicologists, it is possible to enhance intuitive approaches, optimization and design of experiments. Supported in part by NIH Training Grant # ES07157.

1277 DEVELOPMENT OF A PHYSIOLOGICAL MODEL FOR DIMETHYL-ARSINIC ACID (DMA)

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DMA is the major metabolite formed after exposure to arsenic and is also used as an herbicide. It induces lung-specific damage in the form of DNA single strand breaks and DNA-protein crosslinks in mice and rats. We have developed a physiologically-based pharmacokinetic (PBPK) model for DMA in rodents to both predict lung burdens of DMA and as part of a larger effort to develop a PBPK model for arsenic. The compartments in the model include blood (plasma and cellular fractions), lung, liver, kidney and slowly and rapidly perfused lumped tissue groups; DMA transport was modeled assuming blood-flow limited kinetics in plasma. The model incorporates oral and intravenous routes of exposure, urinary and fecal excretion of DMA, first order metabolism of DMA to trimethylarsine oxide in the liver and binding of DMA in blood. The model provided an adequate fit to kinetic data for mice and rats from the published literature, as well as to limited available human data after appropriate scaling. Application of sensitivity analysis to the model indicated that the impact of model parameters influencing DMA renal excretion differed markedly for mice and humans. In mice, renal blood flow was the parameter with the greatest magnitude and time-dependent effect on renal excretion of DMA. In humans, changes in both kidney:blood partition coefficient and net renal extraction coefficient had similar and much greater impact on renal excretion of DMA. Given the significant strain- and sex-dependent differences in glomerular filtration rate and kidney weight that are known to exist in mice, this suggests the need for sex- and strain-specific values for these parameters in PBPK models incorporating renal excretion for this species. This finding also suggests that species-specific application of sensitivity analysis to PBPK models can be an important element in animal extrapolation and risk assessment. (This abstract does not necessarily reflect EPA policy).

1278 SENSITIVITY ANALYSIS OF A STEADY STATE PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR TCDD IN RATS

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TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is an environmental toxicant which exhibits nonlinear kinetics since it induces CYP1A2, a specific binding protein in the liver. The objectives of this study were to develop a physiologically based pharmacokinetic (PBPK) model describing steady state conditions and to make use of sensitivity analysis to assess the impact of different model parameters. The simulated dose response curves for the total body fraction in the liver (FH) and the liver to fat ratio (LTOF) were sigmoidal in shape, exhibiting an inflection point (IP) and a maximum (MAX). The normalized sensitivity coefficients (SCs) for LTOF at MAX for the following parameters: Pf (fat partition), Kb2 (CYP1A2 binding affinity) and BM2i (amount of CYP1A2 induced) had the highest values, indicating that these are critical parameters. For LTOF at IP, the resulting SCs for three other parameters: Kb1 (Ah receptor binding affinity), Kd (the receptor complex DNA binding affinity) and Bm1 (Ah receptor binding maximum) were about half the value for those of Pf, Kb2 and BM2i, therefore these are less critical parameters. In general, the SCs for FH exhibited the same behavior as those for LTOF, but were smaller in magnitude. In order to predict the impact of different TCDD analogs, the sensitivity analysis was repeated after decreasing Kb2 and increasing both Kb1 and Kd. The behavior of the two sigmoidal dose resonse curves was different in that LTOF SCs appeared unaffected by the variations, while the SCs for FH appeared significantly affected by the same changes. In summary, sensitivity analysis provided a systematic approach to assess the impact of different model parameters for TCDD and to predict behavior expected due to changes in parameters describing different analogs. (This abstract does not necessarily reflect EPA policy).

1279 PHYSIOLOGICALLY BASED MODELING OF BUTADIENE AND BUTADIENE EPOXIDE TOXICOKINETICS

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A physiologically based pharmacokinetic model has been developed in rats and mice for butadiene (BD) and its epoxide metabolites, 1,2-epoxy-3-butene (BMO) and 1:2,3:4-diepoxybutane (BDE), by integrating in-vitro metabolism and tissue partitioning data with in-vivo uptake and disposition data. The model contains flow- and diffusion-limited com-partments and describes experimental data from multiple sources and exposure scenarios, lending confidence in the use of the in-vitro metabolic parameters. Incorporation of diurnal glutathione variation allows accurate simulation of glutathione changes observed in mice and rats after nose-only exposures to air (control) and BD.

Due to rapid metabolism in some tissues, significant decreases in BMO and BDE concentrations can occur during the short time required for tissue collection and initial processing. An isolated tissue model, based on in-vitro rate parameters, predicts declines in tissue levels of BMO and BDE subsequent to exsanguination. This model improves estimates of in-vivo dosimetry by matching epoxide concentrations measured after tissue processing with the levels that existed in the tissue at the moment exposure ended.

Human dosimetry data are difficult to obtain, but a rodent model can serve as template for a model that predicts human dosimetry, once it has been validated. A human dosimetry model would make it possible to estimate internal BMO and BDE dosimetry for human workplace exposures. (Supported in part by a grant from the Chemical Manufacturers Association.)

1280 BIOLOGICALLY-BASED MODELING OF INTRACELLULAR ACIDIFICATION OF NASAL EPITHELIAL CELLS INDUCED BY VINYL ACETATE EXPOSURE

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Vinyl acetate (VA) is used in the manufacture of polymers for the paint, adhesives and paper board industries. Chronic inhalation bioassays show that VA causes nasal tumors in rats and olfactory degeneration in rats and mice. Metabolic and inhalation dosimetry studies show that VA is extensively metabolized to acetic acid and acetaldehyde. Acetaldehyde is further oxidized to acetic acid. It is proposed that intracellular accumulation of acetic acid overwhelms cellular buffering capacity and results in cytotoxicity and that this mechanism is integral to the expression of tumors. A biologically-based model was constructed to describe nasal uptake and metabolism of VA and changes in epithelial intracellular pH (pH_i), defined as a function of intracellular $[H^+]$. The source of H^+ is the dissociation of acetic acid formed from VA and acetaldehyde oxidation. H^+ removal is controlled by a composite intracellular buffer (70mM) and a pH-dependent Na^+/H^+ pump. A Hill equation was used to describe the pump. The model was particularly sensitive to the airflow characteristics, carboxylesterase kinetic constants, and the Na^+/H^+ pump parameters. Simulations of 6 hour exposures to 0, 50, 200, and 600 ppm yielded estimates of olfactory epithelial pH_i of 7.4, 7.39, 7.22, and 6.72, respectively. Chronic exposures of rats to these concentrations yielded incidences of olfactory epithelial degeneration of 0, 3%, 67%, and 86%, respectively, suggesting that sustained exposure to elevated H^+ concentration (reduced pH_i) is associated with cytotoxicity. Although there are currently no quantitative data to validate the estimates of epithelial pH_i, the model predictions are consistent with *in vivo* observations of toxicity. These preliminary modeling results support the proposed hypothesis, suggest a biologically-based model for assessing human exposures to vinyl acetate vapor, and provides important insights to future mechanistic research. Supported in part by the Vinyl Acetate Toxicology Group, Inc.

1281 INTERINDIVIDUAL DIFFERENCES IN $^2H_8$-TOLUENE TOXICOKINETICS ASSESSED BY A SEMI-EMPIRICAL PHYSIOLOGICALLY-BASED MODEL

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To explore how interindividual physiologic differences affect the absorption, distribution, metabolism, and excretion of inhaled toxicants, we conducted 33 exposures of 26 male subjects to 50 ppm $^2H_8$-toluene for 2 hr at rest, and collected venous blood samples for 120 hr post-exposure. $^2H_8$-toluene concentrations were measured using a headspace method with cryogenic trapping, capillary gas chromatography, and mass spectrometry. A physiologically-based toxicokinetic model incorporating lung metabolism was used to probe for subject-specific characteristics important to $^2H_8$-toluene disposition. A 10-fold interindividual range in venous concentrations was found. Subject-specific modeling explained 91% of the observed data variability, compared to 53% using literature values for model parameters. Significant interindividual differences in systemic clearance and fit adipose blood flow and extrahepatic

metabolism were found. Adipose blood flow was correlated with the measured fraction of adipose tissue. Body weight, adipose tissue fraction, and blood/air partition coefficient were correlated with terminal half-life, steady-state volume of distribution, and terminal volume of distribution. Extrahepatic metabolism was correlated with systemic clearance and terminal half-life. An increased adipose fraction led to lower blood concentrations up to 8 hr post-exposure. Use of subject-specific model parameters greatly improved model fit and demonstrated interindividual differences in toxicokinetics. Financial support was provided by the Superfund Basic Research program, NIEHS ES 04696.

1282 A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR INGESTION OF CHROMIUM (III and VI) IN DRINKING WATER: VALIDATION WITH HUMAN STUDIES

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A physiologically-based pharmacokinetic model (PB-PK) for trivalent and hexavalent chromium was developed using rodent data scaled up to human parameters for physiology and chromium kinetics. The model was calibrated with human ingestion data on trivalent chromium compounds and prelimnary data from recent human volunteer studies of hexavalent chromium in drinking water. The substantial reducing capacities of gastric fluids and the blood demonstrated in recent studies suggests that drinking water exposures exceeding 10 mg Cr(VI)/day would be required to result in any measurable or substantial distribution of the hexavalent form beyond the stomach. At the Cr(VI) water concentrations examined in our human studies, the PB-PK model predicts systemic uptake and distribution of chromium only in the trivalent form. The model also predicts that high concentrations of Cr(VI) would be required in drinking water to overwhelm the reductive capacity of the stomach and the blood. Since Cr(VI) imparts a bright yellow color to drinking water even at 10 mg Cr(VI)/L, this model suggests that plausible long-term exposures to Cr(VI) in drinking water below this level are pharmacokinetically equivalent to uptake of the more bioavailable forms of trivalent chromium.

1283 A PHYSIOLOGICALLY-BASED PHARMACOKINETIC DESCRIPTION OF BROMODICHLOROMETHANE (BDCM) TISSUE DOSIMETRY AND METABOLISM

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BDCM is a by-product of water chlorination which is carcinogenic in rats and mice. The carcinogenic response induced by BDCM in rodents is likely due to the amount of compound reaching target tissues and the rate of BDCM metabolism to reactive intermediates. To estimate the delivery of BDCM to target tissues following oral gavage, we have developed a physiologically-based pharmacokinetic (PBPK) model for BDCM in the male F-344 rat. The model consists of six compartments (liver, kidney, slowly and rapid perfused tissues, fat and gut). The gut compartment consists of a number of sub-compartments, each described by an absorption constant (Ka; l/hr), a bioavailability term (A; unitless) and a time of emptying (T; hours). Constants for BDCM metabolism were obtained from blood bromide determinations following constant concentration inhalation exposures (50–3200 ppm for 4 hr). Levels of BDCM in tissues and exhaled breath following oral gavage of 50 or 100 mg/kg in corn oil or 10% Emulphor were quantitated using gas chromatography techniques. Parameterization of the oral uptake of BDCM was accomplished by fitting blood and exhaled breath chamber BDCM concentration-time profiles by changing values of Ka, A, and T. BDCM metabolism, assessed by blood bromide production, was described using two saturable pathways ($Km_1 = 0.5$ mg/l; $Km_2 = 5.0$ mg/l; $Vmaxc_1 = 11.0$ mg/hr/kg; $Vmaxc_2 = 2.0$ mg/hr/kg). Model simulations were in good agreement with measured target tissue BDCM levels using oral uptake parameters obtained by fitting exhaled breath curves. This work increases the confidence in the present model structure and values for metabolic constants. This model will help reduce the uncertainties in extrapolations from animal bioassay results following oral gavage with BDCM to the human exposure situation. (This abstract does not reflect EPA policy).

1284 PHYSIOLOGICALLY-BASED PHARMACODYNAMIC MODELING OF ACUTE CARDIOVASCULAR RESPONSE TO REPEATED NICOTINE ADMINISTRATION IN HUMANS

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Tachyphylaxis is a well-characterized physiological response to the effects of many xenobiotics. To better understand this process, a physiologically-based pharmacodynamic (PB-PD) model which includes a negative feedback control system was applied to further describe the previously reported effects of nicotine on heart rate. The PB-PD model was linked to the tissue concentrations predicted from a physiologically-based pharmacokinetic (PB-PK) nicotine model which we have developed. The combined PB-PK/PD model was evaluated by fitting published PK and PD data obtained following two 30-min intravenous infusions of nicotine to humans (Porchet *et. al.*, 1988). After simulating nicotine tissue concentration-effect proterisis, the arterial, heart and brain tissues were the apparent effect sites on heart rate. Another simulation for acute administration of nicotine was run using brain nicotine concentrations as input to the model. This approach helps eliminate apparent tachyphylaxis attributable to distribution delays in tissues. The negative feedback system was modeled to quantify the rate and extent of acute tolerance development and to consider homeostatic mechanisms. Simulation results for heart rate indicated different rates (0.40–0.79 hr^{-1}) of acute tolerance development following different intervals between successive intravenous infusions of nicotine. The combined PB-PK/PD model allowed adequate quantitative descriptions of heart rate changes based on more physiologically relevant mechanisms to predict both PK and PD responses to nicotine. Therefore, the nicotine PB-PK/PD model is a more useful tool to study nicotine's physiological and pharmacological effects.

1285 A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR TOLUENE DISPOSITION IN RATS

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Toluene is an organic solvent widely used in many industries and commercial products. When inhaled, toluene exerts its main toxic effects on the central nervous system (CNS). The objective of this study was to develop and validate a PBPK model for toluene disposition in rat as a basis for correlating toluene concentration in the CNS to observed neurobehavioral effects. The model consisted of the following compartments: brain, liver, lung, adipose tissue, richly perfused tissues and slowly perfused tissues. Physiologucal parameters, metabolic rate constants and chemical specific parameters, such as solubility in blood and selected tissue (except for brain) were obtained for the literature. Brain to blood partition coefficient was measured in homogenates of brain Sprague Dawley rats using vial equilibration technique and was estimated to be 1.1 ± 0.38 (mean $\pm$ sd, $n = 8$). Using these parameter values the model well predicted published data of toluene levels in brain and venous blood of rats after single acute exposure (75, 150, 225ppm, 5 hours; 500 ppm, 4 hours; 2000 and 10000 ppm, 0.5 hours) and after repetitive exposure (150 ppm, 5 hours for 9 days) to toluene by inhalation. This model appear useful for understanding the relationship between brain toluene concentration and toxicological effects and it represents a first step for developing a human model.

1286 PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELING OF CARBON TETRACHLORIDE (CCl_4) TISSUE DISTRIBUTION IN RATS AFTER EXPOSURE BY INHALATION, ORAL GAVAGE AND INTRAGASTRIC INFUSION

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Although CCl_4 metabolism has been extensively studied, there is little information on CCl_4 tissue concentration. Previously, we have developed a single, consistently parameterized PBPK model for CCl_4 to describe arterial blood concentration time-course data in fasted, adult male Sprague-Dawley (SD) rats for three routes and two levels of exposure. The objective of the present study was to characterize CCl_4 pharmacokinetics in the liver and extrahepatic tissues following oral and inhalation exposures with the previously developed

PBPK model. The PBPK model was used to describe CCl_4 tissue and arterial blood concentrations in fasted, adult make SD rats following exposure to 1000 ppm for 2 hr by inhalation or exposure to 179 mg CCl_4/kg given either by oral gavage or 2-hr intragastric infusion. Blood samples (obtained by cardiac puncture) and samples of liver, fat and brain were collected during and after exposures for up to 24 hr and analyzed for CCl_4 concentration by headspace gas chromatography. Based on present data, there was generally good agreement between the model predictions and observed CCl_4 tissue and arterial blood concentrations for all three routes of exposure, except overprediction was noted for liver concentrations following intragastric infusion. CCl_4 tissue and blood concentrations were lower following intragastric infusion than after oral gavage and inhalation exposure. As predicted by the model, CCl_4 concentrations were highest in the fat compartment followed by liver, brain and blood, respectively. In conclusion, our previously developed PBPK model was able to adequately describe CCl_4 blood and tissue concentration time-course data for multiple routes of exposure. (This abstract does not necessarily reflect EPA policy. Supported by EPA Cooperative Agreement CR-820447.)

1287 POTENTIAL ERRORS IN THE USE OF BODY BURDEN CALCULATIONS FOR ESTIMATION OF TOXICOKINETIC PARAMETERS

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The analysis of "body burden" data in toxicology research is extensive as evidenced by reports in the literature. Body burden is the amount of a xenobiotic that is present in the body at a given time. When body burden data are plotted on a semilogarithmic scale as a function of time, important toxicokinetic parameters such as elimination rate constant and disposition half-life may be estimated. This method of analysis is identical in concept to one frequently used in pharmacokinetics, namely the amount remaining to be excreted (ARE) method. Assuming a one-compartment model and first order kinetics, the ARE at time t is calculated using the equation: Xu_∞-Xu, where Xu is the cumulative amount of drug excreted up to time t, and Xu_∞ is the total amount of drug that is excreted through the renal pathway. Xu_∞ is equal to: F'Xo, where X° is the dose, F' is the product of the bioavailability (F) and the fraction of drug excreted into urine (f_{urine}). The semilogarithmic plot of the ARE data versus time is used to estimate values of the elimination rate constant, elimination half-life, and F'. Using simulated data, we demonstrated the errors that result from the incorrect calculation of the ARE. These errors occur when X° is used instead of Xu_∞, or urine is not collected to completion. We developed three methods whereby incorrect ARE data can be analyzed to find accurate estimates of the parameters. Of the three methods we recommend the use of Method 3 (the explicit fitting method). All simulations and optimizations were performed using SIMUSOLV.

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1288 A BIOLOGICALLY-BASED KINETIC MODEL FOR THE ISOLATED PERFUSED RAT LIVER

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The isolated perfused rat liver (IPRL) preparation is a useful tool to investigate the role of the liver in the kinetics of xenobiotics in mammals. This model system is particularly useful because it maintains the liver architecture, as well as the normal route of perfusion. In addition, mechanisms of biliary excretion can be investigated. In order to describe the kinetic behavior of xenobiotics in the system, a generic biologically-based kinetic (BBK) model for the IPRL system has been coded in ACSL11 and an executable program debugged. The model is designed to take into account the following biochemical and physiological processes in the liver: (1) membrane transport, (2) protein binding, (3) metabolism, and (4) biliary excretion. The model corrects for the removal of samples from the system during the course of the experiment. The current version describes the behavior of both the parent compound and one metabolite, assuming that metabolism can be described by a Michaelis - Menten type equation. Additional metabolic pathways can be incorporated into the model as needed. This model can be used to analyze kinetic data for the concentration of the parent chemical and the metabolite in the perfusion medium, in bile and in the liver at the end of the perfusion.

1289 A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL PREDICTING LIVER DOSIMETRY OF KEPONE

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A physiologically based pharmacokinetic (PBPK) model was used to predict the gavage dose of Kepone that will produce the same liver concentration in rats fed with 10 ppm of Kepone for 15 days in diet. For this reason, a previously published model (Bungay et al., 1981), was modified to account for the feeding experiments. Simulations by the modified model indicated that the estimated liver concentration at the end of the 15 day feeding is 80 ppm. Based on the PBPK modeling simulations, this level of Kepone in the liver can also be achieved by the application of a single gavage dose of 16 mg/kg. Furthermore, based on the area under the curve of liver concentration versus time, the model also estimated a period of 8 days after a 16 mg/kg gavage dose to yield the same cumulative dose at the end of a 15 days feeding of 10 ppm Kepone. To validate the model prediction, experiments were designed to collect tissues (blood, liver, and fat) at 2, 4, 24, 192 and 360 hours for the gavage experiment and at 4, 8, 12, and 15 days for the feeding experiment. Ultimately, the model predictions are utilized to study the interactive toxicity of Kepone on CCl_4 by the application of a single gavage dose instead of a lengthy feeding protocol.

1290 A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR PENTACHLOROBENZENE DISPOSITION IN F344 RATS

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As part of an effort to incorporate physiologically-based pharmacokinetics (PBPK) in evaluating target tissue dosimetry of pentachlorobenzene (PeCB), a PBPK model was developed in F344 rats. The model consists of seven tissue compartments (blood, liver, kidney, fat, richly perfused, and slowly perfused tissues) with a multi-compartment description of gastrointestinal absorption. Blood, tissue, and excretory time-course concentrations were utilized to calibrate the model. Based on preliminary data, the kinetics of PeCB appear to be diffusion limited in all tissues with the exception of fat. According to modeling estimates, approximately 38% of the original gavage dose was excreted unabsorbed in the feces while absorption of the dose was occurring in the distal gastrointestinal tract as late as 15 hours post-administration. Time-course analysis of pentachlorophenol (PCP) suggested that less than 1% of PeCB was metabolized to PCP at the end of 72 hours which is consistent with model estimates of approximately 2%. This model will reduce uncertainty in estimating the target tissue dosimetry and allow us to eventually characterize the absorption, excretion, and tissue kinetic parameters of PeCB under the specific experimental conditions association with Ito's Medium-Term Liver Foci Bioassay.

1291 THE USE OF PRECISION-CUT RAT LUNG SLICES FOR STUDYING DRUG METABOLISM

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The suitability of a dynamic lung slice culture system was assessed as an *in vitro* model for *in vivo* drug metabolism. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), a twenty carbon fatty acid that contains a five-carbon ring and is known to be metabolized by lung *in vivo*, was incubated with precision-cut rat lung slices using a dynamic slice culture system. Each incubation consisted of 1.7 ml of a 1 ng/0.6 μCi/ml solution of ^{3}H-$PGF_{2\alpha}$ in Waymouth's buffer fortified with 10% fetal calf serum, and three precision-cut rat lung slices floating on a revolving titanium screen. At 0, 3 or 6 hr after addition of ^{3}H-$PGF_{2\alpha}$, incubation was stopped and the contents of each vial were analyzed for remaining ^{3}H-$PGF_{2\alpha}$ and its metabolites using reversed-phase HPLC with radiochemical detection. Slice viability was assessed by measuring protein synthesis and adenosine triphosphate (ATP) concentrations in lung slices through 24 hr of incubation. $PGF_{2\alpha}$ was metabolized to 15-keto $PGF_{2\alpha}$, 13,14-dihydro-15-keto $PGF_{2\alpha}$, and three unknown minor polar metabolites. These results indicate that $PGF_{2\alpha}$ was metabolized in lung slices by a variety of pathways similar to those seen *in vivo*. Both protein synthesis and ATP concentrations were maintained over the entire 24 hr incubation, indicating that the lung slices were viabile for at least 24 hr, which is a sufficient time for slices to metabolize most xenobiot-

ics. These results demonstrate that the dynamic lung slice culture system is an appropriate *in vitro* model for predicting the *in vivo* pulmonary metabolism of $PGF_{2\alpha}$.

1292 DEVELOPMENT OF DOG MODEL FOR PRECLINICAL SAFETY EVALUATION OF DRUGS TO BE DELIVERED BY EPIDURAL OR INTRATHECAL ROUTES

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To evaluate preclinical safety of spinal drugs, two models using beagle dogs (Rigdland Farms, 10–15kg) employing intrathecal (IT) and/or epidural (EP) catheters were developed and characterized for toxicokinetic and safety evaluations. Implantation is performed under halothane anesthesia using rigid aseptic protocols. For IT, polyethelene (PE) catheters (0.6 mm OD), radiation-sterilized, are inserted from the exposed cisterna magna 40 cm to L1–3. Catheters are fixed and externalized on the lower neck. For EP delivery, PE catheters (0.97mm OD) are placed percutaneously at the L7-S1 interspace 10 cm. Catheter is fixed and externalized on the upper back. Drug delivery is by vest-mounted pumps (Disetronics). In this work, groups of dogs, prepared with EP (N = 10) or IT (N = 4) catheters, received 28 days of continuous infusion of saline (167μl/hr). At day 28, dogs were anesthetized, cisternal CSF sampled and a whole body clearance of blood and fixation performed. CSF protein and glucose levels for all dogs were slightly elevated, but were within normal range. No autonomic/ motor/sensory dysfunction was encountered. A modest local pericatheter fibrotic reaction and a mild meningeal inflammation was noted with the IT/EP catheters. The IT catheter resulted in modest cord compression, but no cell damage or gliosis was noted. These models are thus appropriate for evaluation of IT or EP drug safety.

1293 INTRACEREBROVENTRICULAR ACCESS TO THE RHESUS MONKEY BRAIN FOR SPECIALIZED TOXICITY STUDIES

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Technology and procedures have been developed for targeting, implanting, and verifying the location of catheters implanted in a lateral ventricle of approximately 5 kg rhesus monkeys to support toxicity studies which are now ongoing. A magnetic resonance imaging (MRI) compatible headholder designed based on experience with the unit described by Teale et al. (Teale, P., Reite, M., and Simon, J. (1993) A nonmagnetic stereotaxic head holder for macaque monkeys. *Behav. Res. Meth., Inst. & Comp.*, 25 (4), 468–471) was used to position the monkeys in the knee coil of a GE Signa 1.5 Tesla MRI. MRI under propofol anesthesia was used to scan the brain for abnormalities and target the lateral ventricle. MRI information was used in combination with conventional x-ray images of bony landmarks. During surgery under halothane or isoflurane anesthesia and using individualized coordinates, a catheter was introduced through a bur hole approximately 2 mm in diameter, placed in the lateral ventricle, and affixed to the skull. Confirmation of access to the ventricle included the use of an Imagyn fiberoptic endoscope. Location of the catheter in the ventricle was assessed following injection contrast agent (Isoview 300). Of the 39 animals implanted in this series, 36 had appropriately placed catheters for intracerebroventricular access. Use of multiple techniques has led to a technology for reliably placing intracerebroventricular catheters in rhesus monkeys.

1294 28 DAYS CONTINUOUS INTRATHECAL INFUSION IN CYNOMOLGUS MONKEYS USING AN INDWELLING CATHETER

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Intrathecal infusion via an indwelling catheter is used to administer some compounds clinically. To assess these compounds in preclinical safety evaluation studies, the same route should be used in animals.

To determine the feasibility of intrathecal infusion in cynomolgus monkeys, 6 animals were anesthetized, a needle was inserted in the lumbosacral area and a catheter fed through the needle so that the tip lay in the intrathecal space at the lumbar area. Following removal of the needle, the catheter was sutured in place, passed subcutaneously and exteriorized in the scapular region. The catheter was connected to an external pump using a jacket and tether system which allowed the animal free range movement within the cage. Monkeys received 0.9% sterile saline by continuous infusion at a rate of 0.1 ml/hr for 28 days.

Withdrawal of cerebrospinal fluid and radiography (using an imaging agent) were performed post surgery and preterminally to confirm placement of the catheter. Clinical observations, neurological examinations, bodyweight gains, food intake, ophthalmoscopy, electrocardiograms, blood pressure and clinical pathology revealed no adverse effects. Gross and histopathological examinations after 28 days showed only minor findings which were considered to be a normal tissue response to the presence of an indwelling catheter.

It was concluded that intrathecal infusion in cynomolgus monkeys is a viable route for preclinical safety assessment studies of at least 28 days.

1295 BONE MINERAL DENSITY MEASUREMENT USING DXA IN OVX CYNOMOLGUS MONKEYS

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Dual Energy X-ray Absorptometry (DXA) quantifies the bone mineral density (BMD). The overiectomized (OVX) primate is proving to be a useful model for studying treatment drugs for osteoporosis. BMD is one of the important measurement parameters for osteoporosis. In this study, the changes in BMD were examined in OVX female monkeys.

Bred female cynomolgus monkeys, 9–13 years old, were used. The animals were housed individually under the following conditions: temperature, 26 ± 2°C; humidity, 50 ± 10%; air changes 15 times/h; and a 12h light cycle. The animals were fed 110 g of Harlan Teklad daily. The animals were anesthetized (ketamine), and measurements of BMD were made using the DPX-α (Lunar), according to Hiyaoka's method (1994). To restrict spinal movement animals were placed on a v-mat. The analysis were performed using the Lunar program for human children. The calculations were performed using a mineral bone phantom on lease by the National Institute of Health, Tsukuba Primate Center.

Both sham operated and OVX monkeys were studied, and the OVX monkeys showed low BMD compared to the sham monkeys. In urinalysis and serum biochemistry parameters, comparing OVX and sham monkeys, there were additional differences noted. Therefore, the OVX cynomolgus monkey may be a useful model for human osteoporosis studies.

Hiyaoka A, et al. (1994) Age-related changes in bone mineral density, mean width and area of the lumbar vertebrae in male African green monkeys (*Ceropithecus aethiops*). *Exp. Anim.* 42(2):235–241

1296 THE USE OF PUSH/PULL CANNULATION AS A VIABLE ALTERNATIVE IN MULTIPLE BLOOD SAMPLING

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A method was developed for collecting multiple blood samples without disrupting the pharmacodynamic of the subject. The study consisted of implanting cannulas in the femoral artery and vein of four Fischer rats. Three-way stop-cocks were placed at the end of each cannula. Animals were placed in nose-only holders. A 1 mL whole blood sample was collected from the arterial cannula at the following time points: 0, 5, 10, 15, 30, 45, 60, 90, 120, 180 and 240 minutes. Following each collection time point, an equal volume of whole blood (1 mL) was infused back into the animal via the venous cannula. The donor blood was collected from animals of the same sex, strain and age and crossmatched and pooled in a satellite blood collection system containing Citrate-phosphate-dextrose-adenine (CPDA-I). Blood samples were evaluated for pH, blood gases (pO_2 and pCO_2), bicarbonate, hemoglobin levels, glucose, electrolytes, total protein, lactate dehydrogenase (LDH), creatine kinase (CPK), and blood urea nitrogen (BUN).

1297 A COMPARISON BETWEEN THE KRUMDIECK AND BRENDEL TISSUE SLICERS: ASSESSMENT OF VIABILITY USING CADMIUM AS A MODEL TOXICANT

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The Krumdieck and the Brendel tissue slicers are routinely used to prepare precision cut tissue slices for *in vitro* studies. Although several important differences exist between the two instruments, a direct comparison of relative slice quality has not been reported. Using cadmium ($CdCl_2$) as a model

toxicant, we have evaluated the viability of slices prepared using each of these instruments. In addition, we have compared cytotoxicity assays and the effect of tissue slice thickness on the accurate assessment of slice viability. Tissue slice thickness was determined using a metric gapping gauge/8X eyepiece and histology. Weights of slices (8mm X 20 or 30mg), prepared using each of the instruments, differed by less than 1mg. Following a 1.5h preincubation, slices were placed in Waymouths media and cultured in a roller incubator system. Tissue slices were exposed to increasing concentrations of $CdCl_2$ (0, 1, 10, 100, 500μM) for 12h. Lactate dehydrogenase (LDH) leakage into media increased significantly following exposure to 100 and 500μM Cd in 20mg slices. In 30mg slices, LDH leakage was only increased following exposure to 500μM Cd. Mitochondrial function (MTT) was also significantly decreased in 20mg slices exposed to 100 and 500μM Cd. In contrast, 30mg slices demonstrated this toxicity only at 500μM Cd. Paraffin embedded tissue slices were stained with hematoxylin/eosin and examined. Both instruments produced slices of comparable histology. Furthermore, a notable increase in necrosis at 100 and 500μM Cd was observed. Based on these results, a clear difference in the quality of slices produced by each of these slicers was not apparent. However, tissue slice thickness and the type of assay used to assess viability are critical for accurate interpretation of slice toxicity data.

1298 THE ACUTE TOXIC CLASS METHOD - A VALIDATED ALTERNATIVE TO THE ORAL LD_{50} TEST

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The oral Acute Toxic Class (ATC) method has been validated with 20-30 substances in collaborative studies with 15 participants in 6 countries with the aim to allocate a tested substance to the toxicity classes of the international classification systems currently in use. It is a stepwise procedure with the use of 3 animals of one sex per step. It has been designed with 3 fixed starting doses (25, 200 and 2000 mg/kg). Occasionally, it may be desirable to achieve a further refinement of classification by further testing at additional fixed dose levels of 5, 50 or 500 mg/kg. In general, 2 to 4 steps are necessary to complete a test and to allocate a substance to the classification systems in a similar or even better manner than with an LD_{50} test. The ATC method uses significantly fewer animals than the LD_{50} test, the average being 70% fewer. This also results in substantially fewer moribund/dead animals. The ATC method is based on biometric evaluations and a comparison between the experimental results and biometric calculations revealed no noteworthy deviations. Recently, the Organisation for Economic Co-operation and Development (OECD) has approved the ATC method as an official test guideline.

1299 THE USE OF PROBABILISTIC MODELING TO DETERMINE REENTRY INTERVALS

John H. Ross and Michael H. Dong. *Worker Health and Safety Branch, California Department of Pesticide Regulation, Sacramento, CA*

Reentry intervals are the earliest time at which workers can reenter agricultural fields safely since last pesticide application. These intervals are established by determining the time T at which the absorbed daily dosage ADD in question is expected to be less than or equal to the safe level SL. One common approach to calculating reentry interval is to determine the time at which the dislodgeable foliar residues DFR (to which workers are exposed) will be, as evident from available dissipation curves, low enough to yield the safe dosage ADD_{SL}. Pesticide foliar dislodgeables can be converted to hourly dermal exposure equivalents through use of a transfer factor TF. The resultant exposure is then multiplied by a dermal absorption rate ABS and divided by a body weight BW to obtain the hourly dermal dosage in question. Mathematically the calculation of this reentry time T can be obtained as follows:

$T = \{\ln[DFRT] - \ln[DFR_0]\} \times K^{-1} = \{\ln[((ADD_{SL})(BW))/((TF)(ABS)(hrs/day))] - \ln[DFR_0]\} \times K^{-1}$, where K is the slope of the (natural) log-linear curve specified by the first-order dissipation model for DFR. Extreme single-point values are usually used for one or more of the above input exposure factors. This conservative approach has a major drawback in that the reentry time so calculated often represents a worst-case scenario that rarely, if ever, happens. Probabilistic modeling is a more realistic alternative wherein the probability distribution of the reentry interval is calculated from the full ranges of the individual exposure factors. Using all the available foliar dislodgeable residues and the full range of transfer factors for a particular crop and work task usually reduces the estimated reentry interval at the 95*th* percentile considerably compared to extreme case point estimates. Probabilistic modeling requires a reasonable knowledge of the distribution of the major input variables. Relatively few pesticides have the requisite DFR (for DFR_0 and K) and TF distributions to justify using stochastic modeling. The purpose of this presentation is to compare the reentry intervals calculated from the conventional approach with those from probabilistic modeling, using worker exposures to three pesticides as case studies for work tasks in tree fruit and grapes.

1300 SENSITIVE, ACCURATE QUANTITATION OF RADIOLABELED METABOLITES WITH ELECTRONIC AUTORADIOGRAPHY

L V Clark. *Packard Instrument Company, Inc., Meriden, CT*

The purpose of this study was to validate the use of direct beta detection with the InstantImager® Electronic Autoradiography System for the detection and quantitation of radiolabeled metabolic products separated by thin layer chromatography. The performance is defined in terms of sensitivity and accuracy.

Samples from a typical toxicology studies were quantified using several different acquisition times on the InstantImager to determine thresholds of detection with respect to count time. The identical samples were quantified by tradition liquid scintillation counting methods to determine the accuracy and counting efficiency.

The results indicate that the detection threshold of the InstantImager for ^{14}C is less than 1.0 DPM/mm^2 in 30 minutes and as low as 0.2 DPM/mm^2 in 16 hours. The R_2 values for the linear regression of the CPM results of the InstantImager with data from traditional liquid scintillation counting were 0.99.

Based on these results, the InstantImager performs with superior sensitivity and accuracy for the quantification of radiolabeled material separated by thin layer chromatography. Ease of use and data handling in the form of image files makes this instrumentation particularly useful for the quantitation of radiolabeled metabolites in toxicological studies.

1301 COMPARATIVE KINETIC EVALUATION OF CHOLINESTERASE METHODS IN RAT AND MAN

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We investigated two published methods of Erythrocyte (AChE), and Plasma Cholinesterase (BuChE) activity: DTNB (5,5'dithiobis-2-nitrobenzoic acid, Ellman, GL et.al. Biochem. Pharmac. 7,88,1961), and DTNA (6,6'dithiodinicotinic acid, Brownson, C et.al. Biochem J. 131, 369, 1973). This study examines the hypothesis that a substitution of DTNA for DTNB in the determination of rat and human AChE is a worthy alternative. To test this hypothesis, the kinetics of the two methods were investigated. The results demonstrate that increasing concentrations of DTNB, but not DTNA, significantly increased K_m for the substrate acetylthiocholine and had little or no effect on V_{max} for rat or human AChE. The K_m or V_{max} was not significantly different for rat or human BuChE. DTNB more so than DTNA, caused linear mixed type of inhibition for rat and human AChE. The nature of interaction of DTNB and acetylthiocholine is such that the DTNB method was found to be unreliable for kinetic investigation of rat and human AChE. All the data yielded Hill coefficients close to 1. Conclusion; 1) DTNA is a more efficient coupling agent than DTNB as demonstrated by the higher V_{max}/K_m ratio for the former. 2) DTNB caused greater inhibition for rat and human AChE, than DTNA. 3) The DTNA method has advantages over the DTNB method, in kinetic investigations of rat and human species, and in toxicological studies where risk assessment of insecticide exposure relies on extrapolation of rat data to humans. 4) The practical aspect of the DTNA method is that it can be adapted to most automated instruments which can monitor the change in absorbency at 340 nm away from the hemoglobin peak.

1302 DEVELOPMENT AND UTILIZATION OF LYMPHOCYTE BLASTOGENESIS ASSAYS IN THE AMERICAN ALLIGATOR (Alligator mississipiens)

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We have developed T and B lymphocyte blastogenesis assays in both hatchling and juvenile (2 to 5 feet) American alligators (Alligator mississipiens). Characterization was performed both on whole blood samples and preparations from spleens. Once the assay conditions of mitogen type and concentration, incubation temprature and incubation time were optimized, the technique

was used to evaluate the immune system of hatchling alligators following in ovo administration of endocrine modulating chemicals. The chemicals used were ethynyl estradiol, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin, o,p'-DDE, p,p'-DDE, coumestrol, and indole-3-carbinol. Ethynyl estradiol and 2,3,7,8-TCDD were dosed at levels from 0 to 10 μg/kg egg weight wheras the remaining chemicals were dosed at levels from 0 to 10 mg/kg egg weight. The eggs were dosed by painting the test chemical dissolved in DMSO on the eggshell. Immune parameters were measured on day 21 following hatch. Alterations in response were noted for each chemical, however these were not dose-responsive.

1303 CHRONIC PERCUTANEOUS CATHETERIZATION OF THE JUGULAR VEIN IN MICE

M Piechowiak, H V Mendenhall, S T Cornell. *TSI Mason Laboratories, Worcester, MA.* Sponsor: F. Mecler

Preclinical evaluation of candidate chemotherapeutic agents requires an animal model which will allow for continuous infusion of test articles. The murine species has been used extensively in such studies, providing a rapid and distinct response of selected tumors to therapy. Continuous intravenous infusion in this animal model, however, has been wrought with difficulty. We have developed a catheter/tether system that obviates the need for a protective jacket (a necessity in tumor studies); a surgical technique; and a post-operative regimen that allows for chronic catheterization of the jugular vein in mice as small as 16 grams. The catheter is made of silastic that is 0.635 mm od x about 25 cm long, contained within a 15 cm length of stainless steel tether. A 2.5 cm length of 3.175 mm od silastic tubing covers the distal 5 mm of the tether and 2 cm of the intravenous catheter as it exits the tether. Using an operating microscope, incisions are made directly over the jugular vein and the interscapular area. The tip of the catheter is introduced from the exit site first. The outer silastic tube is secured to the skin at the exit site with two purse string sutures. The jugular vein is mobilized a distance of about 5 mm. A small incision is made in the vein between two ligatures, and advanced caudally 5–7 mm. The previously placed proximal ligature is tied, and the incision closed in a single layer. The catheter is connected to a standard injection swivel, and continuous infusion begun. Post-operatively, the exit site was cleaned daily with povidone-iodine solution. Over one hundred mice have been so prepared and maintained on continuous infusion for periods of up to 14 days.

1304 AN OBJECTIVE MEASURE OF QUALITY OF PERFORMANCE FOR CLINICAL AND FOB EXAMINATIONS IN RATS

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The need for well trained technicians is recognized, but technician competency is virtually always judged subjectively by their supervisors. Although subjective evaluation of performance cannot be replaced, what constitutes satisfactory performance is nebulous and therefore difficult to communicate to trainees and difficult to document. Consequently, a laboratory performance standard, with a clear pass-fail criterion, was developed to address this deficiency. The performance standard was an idealized composite of FOB data from experienced laboratory personnel. The rats were examined in random order, and treatments were either (1) saline, (2) chlorpromazine, (3) atropine followed by physostigmine, or (4) amphetamine. Thus, the performance standard was the pattern of scores generated by the four treatments. After training, each technician was individually tested on four groups of rats similarly treated (random order, blind examination). The pattern of scores generated by the technician was compared to the performance standard by calculating a Pearson's cross-correlation coefficient. An $r > 0.8$ was considered passing. The use of the standard meets several laboratory goals. (1) Good science. Improved training leads to better studies. (2) Documentation of observer competency is a requirement of the USEPA neurotoxicity guideline. (3) Provides improved documentation of training for Good Laboratory Practices (GLP). (4) Provides objective documentation of performance for purposes of personnel management.

1305 A VALIDATION STUDY TO ASSESS THE SUITABILITY OF THE FERRET FOR CONTINUOUS INTRAVENOUS INFUSION TOXICITY STUDIES

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An indwelling catheter to simulate the proposed clinical route is a routine procedure used in safety assessment studies. The purpose of this study was to evaluate effects of the presence of the catheter in the abdominal vena cava and jugular vein in the ferret, as well as to accumulate control data for this laboratory. A medical grade polyurethane catheter was surgically implanted into either the vena cava via the femoral vein or the jugular vein. All animals were infused with 0.9% Sodium Chloride for Injection for 24 hours/day for 14 consecutive days. Infusion rates of 5 and 10 ml/kg/hour were utilized. All animals were equipped with a jacket and tether system, with a pump connected to the outside of the cage. Additional animals that were surgically catheterized but not infused were maintained on a heparin/saline lock for the duration of the study.

No mortality was seen at either infusion rate. Body weights and clinical pathology parameters were not affected by the intravenous administration of saline during the study. Macroscopic findings were consistent with those seen in routine infusion studies performed in this laboratory.

In conclusion, the ferret is considered to be a suitable species for use in continuous intravenous infusion on a subchronic basis.

1306 PRESERVATION OF RAT LIVERS FOR TRANSPLANTATION USING A NEW COLD PRESERVATION SOLUTION WITH PULSATILE PERFUSION

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Because of the critical shortage of donor organs for liver transplantation, the effects of perfusion of various preservation solutions through rat livers during cold preservation was studied. The eventual goal is to prolong the time over which livers can successfully be cold preserved prior to transplantation. Rat livers were flushed, excised and cold preserved up to 72 h using various preservatives, temperatures, pump flow rates and intervals, and gas phases. After preservation, livers were cored and precision-cut into slices which were incubated at 37°C from 4 – 72 h to mimic some aspects of reperfusion. Endpoints for determination of the condition of the liver slices included intracellular K^+ content, ATP levels, lactate dehydrogenase leakage, heat shock protein content and histopathological assessment. It was found that V-7, a new cold preservation solution originally optimized for preservation of tissue slices and now under investigation as a preservative for whole livers, maintained the viability of the livers to a greater extent than did the clinically used University of Wisconsin solution. One difference between the solutions which might account for the improved preservation is the glucose content since it increased glucuronidation and viability when tested as a preservative for liver slices. The conditions under which perfusion of the liver is performed during cold preservation determine whether the outcome is superior to static cold preservation on ice. Supported by the Arizona Elks (PZN), the University of Arizona Foundation (PZN), the University of Arizona Undergraduate Biology Research Program (TL, BDJ, RAC), CIMD #V95UR009 (JBU) and NIEHS Center Grant P30-ES-06694 (JBU, KB).

1307 RAT AND DOG LIVER SLICE VIABILITY IN SURFACE AND SUBMERSION CULTURE

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Since the introduction of precision cut organ slices there has been a proliferation of slice culture techniques for studies in intermediary metabolism, biotransformation of drugs, chemical induced toxicity, in vitro hybridization, etc. It is of great importance that these tissue slices are obtained in dimensions suitable for these types of investigations and of particular interest is the thickness. Ideally, culture conditions should be such that during the time course of the experiment the most sensitive endpoints do not change for the control. Practically however, it may suffice to just maintain the slices unchanged to the endpoints used in the experiment. We have compared a large number of presently used incubation systems which fall into two groups: submersion and surface culture. Rat and dog liver slices were produced using the Brendel/Vitron tissue slicer under oxygenated and ice cold V-7 cold preservation solution. The slices (200 μm) were incubated in Waymouth's medium + 10 % fetal bovine serum containing $NaHCO_3$ and gassed with 95% O_2 and 5% CO_2. Slice viability was assessed for up to 72 hours using ATP content, K^+ retention, protein synthesis, and histology. The results indicate that surface culture systems maintain organ slices in long term culture

at a higher level of viability than any of the submersion systems. Funded in part by NIEHS 05790 (AJG, KB), a research grant from the Arizona Elks (PZN), support from CIMD V95UR009 (JBU), a fellowship from NIEHS training grant ES 07091 (SJH) and by core facilities of the Southwest Environmental Health Sciences Center ES 06694.

1308 COMPARISON OF RAT AND MOUSE BLOOD PARAMETERS TAKEN USING ISOFLURANE ANAESTHESIA VERSUS NO ANAESTHESIA

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For many years it was the practice at IRI to obtain orbital sinus blood samples by the use of ether induced anaesthesia. Ether is now regarded as unacceptable for use in our laboratories for anaesthesia due to the high risk of explosion, so an alternative was required. Isoflurane (Forane, Abbot Laboratories Ltd) was chosen as the best alternative. Isoflurane is similar to Halothane but is completely unmetabolised and unlike Halothane does not induce liver microsomal enzymes.

This study allowed a comparison of the effects on blood parameters in rats and mice of employing the tail vein without anaesthesia with those obtained via the orbital sinus under Isoflurane anaesthesia. A poster presented at the 1995 International Congress of Toxicology in Seattle compared the differences produced by anaesthesia induced by ether and isoflurane.

The results of the two methodologies used in this investigation for obtaining blood samples show that there is essentially no difference in the results obtained by either method on the major haematology and plasma biochemistry parameters of both rats and mice.

1309 DETERMINATION OF BLOOD:AIR PARTITION COEFFICIENTS FOR POORLY SOLUBLE CHEMICALS

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Due to the difficulty in measuring partition coefficients for poorly soluble chemicals in blood, an alternate to the well-established vial equilibration method was devised. In the vial equilibration method, chemical headspace concentrations in reference vials and test vials were compared to determine the blood:air partition coefficient. When the resulting blood:air partition coefficient was less than one, often these values were compromised by large standard deviations. This phenomenon is true for Halon 1301 and some of its potential replacements. In the method described here, a system was assembled in order to obtain a more direct measurement. Approximately eight milliliters of heparinized rat blood was placed into an exposure flask and exposed to vapor of the chemical of interest at a rate of 30 ml/min. Microdialysis probes were used to determine when the system reached equilibrium. Every 30 minutes, the exposure concentration was analyzed 3 times by gas chromatography. Once the system attained equilibrium, nine 50 µl blood samples were drawn and placed into 12 ml headspace autosampler vials. The vials were heated at 55°C for at least 30 minutes before the headspace was analyzed by hand injections on a gas chromatograph. All chemicals studied had a boiling point less than 50°C to ensure that all of the chemical was driven into the headspace. The blood:air partition coefficients were calculated from the blood headspace concentration and the exposure concentration data. Results indicate that this modified version of the vial equilibration technique yields suitable blood:air partition coefficients for chemicals that are poorly soluble in blood.

1310 THE USE OF THE "MURS GUN" AS A POWDER AEROSOL DELIVERY SYSTEM TO THE BEAGLE DOG

R Labbé, C Banks, C J Perkin, *Bio-Research Laboratories Ltd., Senneville, PQ, Canada.* Sponsor: B Broxup

The Multi-Unit Revolving System (MURS Gun) was developed at these laboratories for administration of powder aerosols to beagle dogs.

The device is used, in conjunction with an appropriate dog mask and a compressed air source, to allow administration of the test material into the dog's mouth. The unit can be easily disassembled for maintenance and cleaning and the metal components contribute to its durability. The efficiency of the system, which is in part dependent on characteristics and the quantity of material to be delivered, is approximately 50% for lactose. The MURS Gun can be calibrated to give mg/burst or total weight. This system provides an accurate way to deliver quickly and simply a wide range of dose levels on short and long term studies.

As part of the system validation, gravimetric assessments (using glass fiber filter papers) and particle size analyses (using the Andersen 1 ACFM Cascade Impactor) were performed and demonstrated good reproducible results. The system was subsequently successfully used on 28-Day and 6-month studies.

1311 AN INVESTIGATIVE STUDY TO ASSESS THE SUITABILITY OF THE FERRET FOR SUBCHRONIC INTRAVENOUS INFUSION TOXICITY STUDIES

S W Fennell, J de Roode, G Washer, C J Perkin. *Bio-Research Laboratories Ltd., Senneville (Montréal), Qc, Canada.* Sponsor: B Broxup

Continuous intravenous infusion via an indwelling catheter is frequently used in preclinical safety evaluation studies to mimic the proposed clinical route. To determine the feasibility of intravenous infusion in ferrets, a medical grade silastic catheter was surgically implanted under general anesthesia in the abdominal *vena cava* via the femoral vein. Animals were infused with saline for 24 hours a day for 28 days at a rate of 5 mL/kg/h using a specially designed jacket and tether system. Clinical observations, body weight gains, food intake and clinical pathology revealed no adverse effects. Gross and histopathological examinations after 28 days showed only minor findings which were considered to be a normal tissue response to the presence of an indwelling catheter and within the range of changes seen in more conventional species dosed via this route. It was concluded that the ferret is a suitable non-rodent species for use in subchronic intravenous infusion studies via an indwelling catheter with only minor changes consistent with the presence of a foreign material.

1312 A FEASIBILITY STUDY OF REPEATED INTRATRACHEAL ADMINISTRATION OF NORMAL SALINE IN THE NEW ZEALAND WHITE RABBIT

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The intratracheal route is a selected route of administration in preliminary, short-term pre-clinical inhalation toxicity studies when test article availability is a limiting factor. This route is also used in screening tests for the delivery of gene therapy drugs.

To determine the feasibility of multiple dose administration via the intratracheal route, 2 male and 2 female New Zealand White rabbits (Oryctolagus cuniculus) received 4 mL of 0.9% saline USP for injection on 5 alternate days. Each dose was administered to sedated animals at a rate of 1 mL/minute using a Penn Century Microsprayer. There were no deaths or changes in body weight or food consumption. Gross and histopathological examinations revealed no abnormal findings that could be attributed to the dosing procedure. This study demonstrated the feasibility of repeated intratracheal administration in the New Zealand White rabbit.

1313 DETERMINATION OF DOMOIC ACID AND KAINIC ACID IN PLASMA AND BRAIN TISSUE USING A FLUOROMETRIC HPLC ASSAY

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A sensitive method for the determination of the seafood neurotoxicant domoic acid (DOM) and the structurally related neurotoxicant, kainic acid (KA) has been developed using a fluorometric HPLC assay. Plasma samples (25 µl) were added to the internal standard dihydrokainic acid (DHKA), acidified with HCl, and drawn through a C18 BondElute column. Brain tissue was homogenized in 3% TCA, 200 µl (20 mg) mixed with DHKA, and applied to the C18 column. Samples were washed with buffer and hexane and eluted with acidified methanol. Extracts were evaporated, reconstituted, and derivatized with 9-FMOC. Chromatography was performed using a Supelco LC-18 column, eluted with 38% acetonitrile in phosphate buffer, pH 2.1 and detected at $\lambda ex = 264$ nm, $\lambda em = 313$ nm. The quantitation limit for DOM is 15 ng/ml using 25 µl plasma and 40 ng/g using 20 mg brain tissue. The quantitation limit for KA is 31 ng/ml in plasma and 40 ng/g in brain tissue. The sensitivity and small sample size make this an excellent method for the quantitation of these neurotoxicants in plasma and tissue of laboratory animals where sample volumes may be limited.

1314 A COMPUTERIZED IMAGE ANALYSIS SYSTEM VALIDATED FOR USE IN GLP OCULAR TOXICITY STUDIES

J N Norton, K L Hall, W D Martin and R B Hackett. *Alcon Laboratories, Inc., Fort Worth, TX.* Sponsor: D P Rodeheaver

Computer systems are an integral component in the conduction of toxicity studies under GLP regulations. A validation study was conducted to assure the quality and integrity of generated data from the Bio Optics Image Analysis System (BOIAS). The BOIAS analyzes photographic images from either *in vitro* corneal perfusion assays or *in vivo* specular microscopy, which provides a morphometric endpoint to assess the toxicity of ophthalmic drugs and devices on the corneal endothelium. An image is "snapped" by the BOIAS software, saved on a 270 MB cartridge, and then retrieved for subsequent analysis, e.g., cell density and shape, and Coefficient of Variation. The functionality of these tasks in the BOIAS was tested in the validation study and its susceptibility to errors determined. Data from a GLP three month ocular toxicity study in rabbits were used for the BOIAS validation study and statistically compared to data previously analyzed at a contract laboratory. Results established that the BOIAS produced analysis data that were acceptable for GLP-regulated studies. The initial validation of the BOIAS for use in the analysis of corneal endothelium and a subsequent validation due to optimization by the computer operator and upgrading of the hardware were considered successful.

1315 DEVELOPMENT AND APPLICATION OF A METHOD FOR DETECTION OF LOW LEVEL VOLATILE ORGANIC COMPOUNDS (VOCs) IN BIOLOGICAL SPECIMENS

N J Andersen and C L Waller. *U.S. EPA, Research Triangle Park, NC.* Sponsor: Linda S. Birnbaum

VOCs are by-products of drinking water disinfection processes and exposure to VOCs via inhalation, ingestion, and absorption has become a major health concern. Physiologically based pharmacokinetic (PBPK) models have been developed for rodents to characterize the uptake, disposition and elimination of VOCs. Typically, development and validation of PBPK models requires experimental dosimetric information from a variety of biological matrices following *in vivo* exposures. Tissue analysis has been a challenging task using conventional analytical chemistry techniques (GC/ECD, etc.) since considerable quantities of these VOCs are lost to evaporation, limits of detection are not sufficient for low level exposure analysis, and homogenization and extraction techniques are inefficient. A sensitive and elemental-specific method for analyzing halomethanes from blood, urine, liver and fat is presented. Gas chromatography/atomic emission detection (GC/AED) coupled with purge-and-trap sampling, thermal desorption, and cryofocusing of analytes provided low parts-per-trillion level of detection for halomethanes with recoveries ranging from 95 – 102%. Limits of detection were determined to be: chloroform ($CHCl_3$), bromochloromethane, and bromodichloromethane - 100 pg, dibromochloromethane - 150 pg, and methylene chloride - 200 pg. Simultaneous detection of $^{13}CHCl_3$ and $^{12}CHCl_3$ from biological specimens was developed without compromising sensitivity or recovery. Characteristic emission spectra distinguished ^{13}C and ^{12}C without cross-reactivity. In conclusion, a method has been developed to analyze VOC concentration and disposition in biological matrices which can be used to develop and validate PBPK models for low level exposures. (This abstract does not necessarily reflect EPA policy).

1316 CAN THE HITACHI 911 AUTOMATED ANALYZER BE USED TO MEASURE CHOLINESTERASE (ChE) ACTIVITY IN TISSUES FROM CARBAMATE-TREATED ANIMALS?

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Previously we have noted that when using a spectrophotometric method to measure ChE activity in carbamate-treated tissues precautions must be taken to limit the reactivation of the inhibited ChE (Nostrandt *et al.*, 1993 *Fund. Appl. Toxicol.* **21**: 196–203). Many testing laboratories use automated instruments to measure ChE activity; instruments which usually employ a spectrophotometric method. To date, there has been no systematic investigation of the validity of the ChE data obtained from carbamate-treated tissues using these automated instruments. The purpose of our study was to compare the ChE data obtained using an automated analyzer with that obtained using a radiometric assay (i.e., the optimal method for unstable inhibitors). Using both the Hitachi 911 automated analyzer and the radiometric method, ChE activity was measured in whole blood and brain tissue taken from rats treated with various dosages of carbaryl (n = 6 control, n = 19 treated). Significant reactivation occurred using the automated instrument. In the brain tissue, ChE activity of the treated tissues was as much as 15% higher using the automated method than using the radiometric method. In the whole blood, there was a 20–30% reactivation using the automated method. Further, detailed analyses indicate that this reactivation is due to the required dilution of the tissue sample for automated analysis as well as the length of the preincubation. Users of the automated instruments must be aware of the possible limitations of these instruments in analyzing ChE activity in tissues from carbamate-treated subjects.

1317 MICRODIALYSIS - A "REAL-TIME-TOOL" TO CORRELATE DRUG LEVELS AND DRUG RESPONSES: LESSONS FROM STUDIES WITH AMPHETAMINE (AMPH)

P Clausing and J F Bowyer. *Division of Neurotoxicology, NCTR/FDA, Jefferson, AR.* Sponsor: W Slikker Jr

To study dose-response relationships, traditionally a series of "snapshots" is taken by sacrificing animals at multiple time points to compare drug levels with drug responses in target areas. In contrast, microdialysis allows for the continuous analysis of drug, as well as amino acid and neurotransmitter levels, combined with behavioral and physiological measurements over many hours. Using this approach, conclusive evidence was provided that differences in drug kinetics are **not** responsible for the occurrence of behavioral and neurochemical "nonresponders" to AMPH (1x2.5 mg/kg sc), because these rats exhibited the same extracellular striatal levels of AMPH (peak levels: 512 ± 88 nM in dialysate, equal to 5.1 μM "true" extracellular levels for both groups together). Also, it was shown that **lack** of neurotoxicity subsequent to 4x15 mg/kg AMPH at 10°C ambient temperature (as compared to **occurrence** of neurotoxicity after 4x5 mg/kg AMPH at 23°C) was not due to reduced drug levels in target sites. The existence of a time-delayed relationship between striatal AMPH levels, locomotor activity and brain temperature was demonstrated, whereas the dopamine increase in response to AMPH was shown to be immediate. In conclusion, microdialysis combined with HPLC and physiological techniques provides a powerful tool for the "on-line" assessment of neurotoxic effects and toxicokinetics.

1318 CHRONIC CATHETERIZATION OF THE LATERAL VENTRICLE IN CYNOMOLGUS MONKEYS

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We have developed a non-migrating catheter system and simple surgical technique for chronic ventricular catheterization in monkeys. The 7 Fr silastic catheter is 20 mm in length with a rounded tip and no perforations. It is connected to a 6 mm x 20 mm silastic base with a side port. The animals are prepared for aseptic stereotaxic surgery. A 5–6 cm midline incision is made, and the temporal muscles on one side are retracted laterally. A 4 mm hole is drilled 13 mm anterior to the ear bars, and 1–2 mm lateral to the midsagittal plane. The dura is opened, and a 10.0 μL Hamilton syringe, with a trimmed 14 gauge iv catheter over its needle, is lowered into the brain until cerebrospinal fluid can be easily aspirated. The depth from the surface of the brain is recorded, the length of which is then marked on the iv catheter. The proximal end of the ventricular catheter is trimmed to this length. The iv catheter is then lowered into the lateral ventricle over the needle of the Hamilton syringe. Its presence in the ventricle is confirmed by gravitational flow of saline. The Hamilton syringe and iv catheter are removed, and the ventricular catheter immediately replaced in the formed tract. The catheter's base is immobilized to the skull with suture to the periosteum. The cap can then be attached to either a vascular access port, or to a percutaneous catheter. The postoperative position of the catheter within the lateral ventricle is confirmed by radiography following the injection of suitable contrast media. We have successfully implanted the lateral ventricle and continuously or intermittently infused test materials for as long as one month.

1319 SPECIFICITY OF ANTIBODIES (Abs) AGAINST RODENT TGF-α PROTEIN

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TGF-α is a mitogenic polypeptide that is postulated to cause cell transformation by interacting with target cells in an autocrine fashion. Because of its importance in tumorigenesis, studies have been focusing on the localization

of TGF-α in both normal and cancer cells. In previous studies, we found that the immunohistochemical distribution of TGF-α varied in rodent tissues depending on whether a monoclonal or polyclonal Ab was used, suggesting that the specificity of anti-TGF-α Abs differed significantly. To address this issue, we compared the specificity of two representative Abs that have been widely used for detecting rodent TGF-α. In a competition study, the Abs were incubated with 10, 50 or 100 fold excess synthetic rat TGF-α 34–50 overnight at 4°C and used for staining of rat and mouse kidneys and/or uterus. The results revealed that one of the Abs, anti-rat TGF-α polyclonal Ab, was neutralized by a 10 fold excess of the peptide, while the other, anti-human TGF-α monoclonal Ab was not absorbed by the peptide up to an amount of 100 fold. Western blotting analyses showed the anti-rat TGF-α polyclonal Ab reacted with both human and pure rat TGF-α. However, the anti-human TGF-α monoclonal Ab showed no cross-reactivity with pure rat TGF-α, although the Ab reacted with mouse protein extracts other than TGF-α from kidneys and uterus; pure human TGF-α; and the molecular marker, bovine carbonic anhydrase. These data indicate that anti-human TGF-α monoclonal Ab does not recognize rodent TGF-α protein at least under the conditions of our study, and suggest that distribution of TGF-α in rodent tissues may need to be reexamined using an Ab that has been proven to have specificity for rodent TGF-α.

1320 ASSESSMENT OF HEAVY METAL ACCUMULATION IN RENAL TISSUE USING MICRO-PROTON INDUCED X-RAY EMISSION (μ-PIXE)

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Heavy metals are common constituents found in industrial refuse stored at disposal sites. We have been successfully using a multi-element analysis technique known as proton induced x-ray emission (PIXE) to conduct heavy metal (Hg, Cd, Cr, As) toxicity studies using precision cut rabbit renal cortical slices and tubule suspensions. Methods involve placing prepared samples in the path of a 2 MeV proton beam and the resulting discrete x-rays subsequently recorded. Beam diameters are 4000 μm, and allow an overall concentration of the metal(s) of interest in the sample to be determined. Current studies being conducted in our laboratory are concentrating on using a modification of this technique (μ-PIXE). This method can produce a proton beam as small as 1 μm in diameter, and includes the ability to raster this beam over an area of up to 125 μm x 125 μm. Analysis by μ-PIXE can be performed on areas of the nephron to pinpoint metal concentrations and identify trends in this susceptible structure. Recent improvements now allow the generation of two separate element maps simultaneously, providing the ability to use an internal standard (such as Fe) while analyzing for a metal of interest to assist in quantification or examine the interaction of two metals at the cellular level. The combination of small beam size, high resolution, and rastering ability makes μ-PIXE a powerful tool for investigating both the local and overall impact of non-endogenous metals in the kidney. (NIH P42 ES 04940).

1321 CLINICAL LABORATORY AND PATHOLOGY COMPARISONS OF THE Crl:CD® BR (CD) AND Hsd:Spregue Dawley® SD® (SD) RAT

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We previously reported higher survival rates and lower total palpable mass incidence, body weight and food consumption in the SD rat in a two-year study. In this phase, we compared clinical laboratory and histopathology parameters in the CD and SD rat. Clinical laboratory differences included lower WBC, RBC, and platelet counts in the SD rats. MCV, MCH, MCHC and % eosinophil values were lower in the CD rats. In the SD rat, progressive nephropathy was more prevalent and was characterized by increased urinary protein and volume, blood urea nitrogen and creatinine. Sequelae to renal disease included hyperplasia of the parathyroid glands, systemic arterial and gastric mineralization, and fibrous osteodystrophy. Microscopic findings with an increased prevalence in the CD rat included pituitary and mammary gland tumors, progressive cardiomyopathy, and various hepatic and pulmonary findings. The total number of animals with neoplasms was similar in the CD and SD rats, however, the total number of neoplasms was higher in the CD rats. In conclusion, age associated microscopic lesions appeared at a higher frequency in the CD rat, however, the SD rat had an increased propensity for spontaneous renal disease.

1322 A SAFE, FACILE AND EFFICIENT METHOD FOR THE INTRA-HEPATIC INJECTION OF TEST ARTICLES IN MICE

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Gene therapy is moving toward wide acceptance as a viable therapeutic option. Accordingly, multiple companies are developing both viral and non-viral gene medicines, which hopefully in the near future will be used like the conventional pharmaceuticals that are currently widely available. A common request as a component of many studies required for the safety and efficacy assessments of new gene medicines is the intra-hepatic administration of test articles. We would like to report the development of a safe, efficient, rapid, painless and non-invasive method for the intra-hepatic parenchymal administration of test articles. Procedures will be described which permit the comfortable restraint and optimal positioning of animals along with the adequate transcutaneous visualization of the left lateral lobe of the liver (regardless of skin color). The procedure was validated in over 250 mice using a microsyringe and variable amounts of a 1% solution of Evans Blue dye in 0.9% saline. The hepatic uptake of dye was rapid and complete. Gross necropsy of treated animals revealed no morphological distortion of the liver and on cut surface only a very small focal hematoma at the deepest point for the site of each injection. Histopathological evaluation of these livers revealed for each injection site the presence of only a small focus of hemorrhage, which was surrounded by a thin narrow band of degenerated and necrotic hepatocytes. The hepatic parenchyma surrounding each of these areas was devoid of any change. Similarly, clinical chemical and hematologic profiles for the animals were normal. This procedure is easily applied to rats and guinea pigs.

1323 A METHOD TO ASSESS MOTIVATIONAL FACTORS IN COMPLEX NEUROBEHAVIORAL PERFORMANCE DEFICITS INDUCED BY TOXIC EXPOSURES

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Sponsor: D Dodd

Commonly-used neurobehavioral assessment batteries (*i.e.*, the Functional Observational Battery or NCTR Operant Battery) seldom consider motivation as a factor in complex performance deficits observed during or after some toxic exposures. The Navy Neurobehavioral Toxicity Assessment Battery (NTAB) provides a methodology for factoring motivational deficits from those due to failures in sensory perception, motor system integrity, or higher cognitive processibg and decision-making, or from physiological irritancy. Rats were highly trained on both fixed ratio (FR-30) and progressive ratio (PR-3) operant schedules. Subjects were then repeatedly exposed during performance to atmospheres containing progressively-increasing concentrations (5,000–180,000 ppm) of ozone-depleting substances (ODSs) CFC-12 or Halon-1211™, or non-ODS replacement (ODSR) HFC-134a. It was clearly shown that performance on the PR-3 task, wherein rats must increase work level throughout each exposure to gain the same food reward, declined more rapidly than on a task (FR-5) where "cost of reward" remained constant. Sensory perception failure, physiological irritancy and nausea were eliminated as factors in the observed deficits. Because performance of FR-5 and PR-3 tasks in room air resulted in almost identical numbers of barpresses, it was assumed that cognitive and motor capacities underlying either performance were similar. Thus, it appears that brief exposure to moderate, but not lower concentrations of CFC-12, Halon-1211™ or HFC-134a results in performance deficits that reflect, at least in part, impairment of motivational systems. It is suggested that motivational factors be considered in existing, proposed and future assessment measures of toxically-induced neurobehavioral deficits.

1324 A NOVEL MODEL TO ELUCIDATE MOLECULAR MECHANISMS OF TOXIC AGENTS

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It is becoming increasingly difficult to reliably extrapolate low-level effects of environmental toxins from mega-doses administered at the organismal level. An appreciation of this problem has led to a call for development of sensitive molecular strategies for assessing the effects of environmental toxins, particularly those that relate to stimulation of cell proliferation. Here we describe a novel strategy for monitoring in single, living cells the activity

of a gene which is relevant to carcinogen-induced proliferation. A plasmid containing the peroxisome proliferator-responsive element (PPRE) inserted upstream of a luciferase reporter gene was used to transfect primary hepatocytes from B6C3F1 mice. Twenty-four hours later, hepatocytes were exposed to luciferin following permeablization with DMSO. Photonic emissions, reflective of PPRE-driven luciferase production, were quantified in "real-time" by use of a Zeiss Axioskope in series with a VIM Photon Counting Camera/ ARGUS-50 Image Processor (Hamamatsu). When 31 cells (4 separate experiments) were studied in this manner, we found that the signal to background ratio was 38.9 ± 6.7 (mean ± SEM). To put it in terms of real events (signal minus background counts), we detected on average more than 15 photonic emissions per second per cell. These results demonstrate that expression of a physiologically/ toxicologically relevant gene can be quantified with very high sensitivity in living hepatocytes. Because the experimental protocol for photonic measurements does not compromise hepatocyte viability or function, this method is currently being used to make multiple, "real-time" measurements of gene expression, with each cell acting as its own control, thereby providing a novel model for elucidating the molecular mechanisms of toxicity. (Supported by DOE grant DE-FG01-92EW50625).

1325 CONTINUOUS INTRAVENOUS INFUSION IN GENERAL AND DEVELOPMENTAL TOXICOLOGY STUDIES

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The procedures used in continuous intravenous infusion studies are usually relatively innocuous but nonetheless, complications associated with the presence of an indwelling catheter or with the infusion system itself may, under some circumstances, have an impact on the outcome and interpretation of the study. Our data generated over five years with a variety of test and control articles under various infusion regimes for periods of up to six months have shown general effects in the rat, rabbit, dog and monkey which have resembled those seen clinically in humans treated in the same way. These effects include minor changes in blood chemistry and haematology resulting from the artificial increase in blood volume, extramedullary haematopoiesis, local infection, thrombus and thromboemboli formation and local irritation of the vein causing thrombophlebitis. In addition, in embryotoxicity, fertility and peri- and post-natal studies (segments I, II & III) our studies show that the volume that can be infused is limited by adverse influences on embryo-foetal development at high infusion rates. In this respect dextrose solution is better tolerated than saline. Therefore, in comparison with conventional methods of administration, the initiation and monitoring of a continuous intravenous infusion study requires more attention, and the interpretation of the findings is more reliant upon available historical background data.

1326 EVALUATION OF THE SPRAGUE DAWLEY® RAT AS AN ALTERNATIVE MODEL FOR THE SUBCUTANEOUS IMPLANTATION ASSAY

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The intramuscular implantation test is routinely used for evaluation of plastics and other polymeric materials that may come into contact with living tissue. Strips of these materials are implanted into the paravertebral muscle of healthy, adult male rabbits via a 15 gauge needle with a stylet. Due to the physical characteristics of various materials (e.g., size, composition, flexibility, etc.), intramuscular implantation may not be possible. Subsequently, subcutaneous implantations in rabbits have been performed as an alternate method. This procedure produced results comparable to expected intramuscular implant results, but required more time and was not considered cost effective. Therefore, an alternative animal model using the Sprague-Dawley® rat has been established in our laboratory. A historical data set comparing intramuscular and subcutaneous implantation in the rabbit and subcutaneous implantation in the rat was compiled. A USP negative control plastic reference standard and an in-house positive control reference standard were used. These materials were intramuscularly implanted into rabbits as described above. Subcutaneous implants in the rats and rabbits were accomplished by placing a sterilized sample into separate subcutaneous pockets on the dorsal surface of each animal. All animals were evaluated at 7 days following the implant procedure. Implant sites were grossly and/or microscopically examined for mechanically induced trauma, capsule formation and the presence of apparent necrotic lesions. There were no observed differences in the average scores between intramuscular (rabbit) or subcutaneous (rabbit or rat) implantation sites for both positive and negative control materials. For the materials tested, rabbit intramuscular and rat subcutaneous implantation procedures were determined to be comparable indicators of localized irritation. Due to this comparability, the rat subcutaneous model is considered a viable alternative model.

1327 IMMUNOHISTOCHEMICAL ANALYSIS OF ASTROCYTOSIS IN SCRAPIE-INFECTED HAMSTERS

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Astrocytosis is a characteristic of scrapie, Alzheimer's disease (AD) and many neurotoxic lesions. Both the abnormal amyloid proteins, "PrP^{Sc}" in scrapie and "β/A4" in AD, can be considered as "endogenic" neurotoxins. One issue is whether the astrocytosis represents increased size or function of individual astrocytes, or, if there is an actual replication of astrocytes. In our study, we used an antibody against proliferating cell nuclear antigen (PCNA) as a determinant of cell replication, and antibodies against basic fibroblast growth factor (bFGF), acidic fibroblast growth factor (aFGF), s100β, and glial fibrillary acidic protein (GFAP) to study the possible mechanisms of astrocytosis in scrapie-infected hamsters. Female, weanling Syrian hamsters, strain LVG/LAK, were injected intracerebrally with scrapie strain 139H or 263K or with normal hamster brain homogenates. Paraffin sections revealed that the intensity of the bFGF, aFGF, S100β and PCNA immunostaining in hippocampus, thalamus, hypothalamus and cortex of 139H- and 263K-infected hamsters was greater than in controls. Furthermore, these proteins were co-localized with GFAP immunostaining in brain cells. Our results suggest that: (1) the astrocytosis seen in scrapie-infected animals is, at least in part, due to actual replication of astrocytes in these hamsters; and (2) the astrocytes can produce and/or absorb bFGF, aFGF and S100β during scrapie infection, which suggests a "snowball effect", that is: these cytokines in astrocytes might play an important role in scrapie astrocytosis.

1328 ALTERATIONS IN PROTEIN KINASE C (PKC) ACTIVITY AND LEVELS OF ISOZYMES IN THE HIPPOCAMPUS OF RATS DEVELOPMENTALLY EXPOSED TO LEAD (Pb)

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We have previously shown that developmental exposure to Pb resulted in increased levels of [^{3}H]-phorbol-12, 13-dibutyrate ([^{3}H]-PDBu) binding, but decreased PKC activity in the hippocampus of postnatal day (PN) 28 rats (Farmer and Guilarte. *The Toxicologist* 15(1): 259, 1995). To further investigate these findings, we have performed a study on the developmental profile of PKC levels using [^{3}H]-PDBu binding and PKC activity in hippocampal subcellular fractions from control and 750 ppm Pb-acetate exposed rats. Our results indicate significantly higher levels of [^{3}H]-PDBu binding in cytosolic fraction from postnatal day (PN) 28, 35, and 56 Pb-treated animals, while increased binding in the membrane fraction were found at PN14, 21, 28, and 35. PKC activity in the same subcellular fraction was measured using the PKC-specific substrate $Neurogranin_{28-43}$. Analysis of variance indicated significant treatment x age interaction ($F_{1,4} = 5.70$; $p = 0.002$) in the membrane fraction. Mean comparisons revealed significantly lower levels of PKC activity in hippocampal membrane fractions from PN14, 21, and 28 Pb-exposed rats. Lower levels of PKC activity in cytosolic fractions were only measured at PN28.

To determine whether the increases in [^{3}H]-PDBu binding were due to changes in specific PKC isozymes, we performed immunoblot analysis using antibodies to PKC-alpha, beta, and gamma in PN28 rats. Significantly higher levels of all PKC isozymes were measured in cytosolic and membrane fractions of Pb-treated rats (except in cytosolic PKC-alpha) at this age. Taken together, the alterations in PKC activity and isozyme levels due to chronic Pb exposure during development may profoundly impact physiological processes in the rat hippocampus. [Supported by grants ESO6189, ESO3819, and ESO7141].

1329 ALUMINUM, IRON AND MANGANESE IN THE MILK OF LACTATING MICE FED A HIGH ALUMINUM DIET AND IN THE BRAINS OF THEIR PUPS

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Interference with essential trace element metabolism is one potential mechanism of metal toxicity. Previous studies have demonstrated altered neurobe-

havioral performance and lower tissue concentrations of iron (Fe) and manganese (Mn), but not aluminum (Al), in weanling mice whose dams were fed high dietary Al. Lower Fe and Mn could be due to lower milk content, or reduced absorption or retention of Fe and Mn by Al exposed pups. In the present study, Swiss Webster mice (n = 11/group) were fed control (7 μg Al/g) or High Al (1000 μg Al/g) diets. Milk and pup tissue trace elements were determined by ETAAS at birth, 3, 7, and 12 days of age, the period during which nutrients are obtained exclusively from milk. Milk was obtained from the stomach of pups who nursed their dams after a 4 hour separation. Dam and pup weights were not affected by dietary Al. Milk Al concentrations were significantly higher in the High Al diet group than controls at 12 days of age only and milk Fe and Mn concentrations were not affected by Al diet. Brain Al was significantly higher in the High Al group than controls at birth only and followed a developmental time course similar to brain Fe, decreasing markedly from birth to 3 days of age in both groups. In contrast, brain Mn rose from birth to 3 days of age in both groups. High dietary Al is not likely to alter Al, Fe and Mn content of milk during early lactation. Supported by ES04190.

1330 RATS THAT HAVE A HYPERTHERMIC RESPONSE FROM FENFLURAMINE (FEN) EXPOSURE EXHIBIT GREATER SEROTONIN (5-HT) DEPLETION

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Fen, a widely used anorectic agent, and (+) Fen, under current investigation for use in the U.S.A., can alter the regulation of body temperature in rats and can deplete 5-HT in rat brains due to Fen toxic insult. We hypothesized that hyperthermia induced by Fen exposure potentiates 5-HT depletion in the manner that hyperthermia potentiates MDMA (methylenedioxy-methamphetamine) neurotoxicity. Six-month old male Sprague-Dawley rats were injected (s.c.) with 3 doses of 5 mg/kg (+) Fen or saline at 2 hour intervals at an ambient temperature of 28°C to enhance a hyperthermic response. The animals were sacrificed 3 days after dosing and 5-HT levels in the striatum (str), frontal cortex (fc) and hippocampus (hipp) were analyzed using HPLC. Animals were categorized as hyperthermic if their rectal temperature exceeded 39°C. Groups were analyzed as control, ≤39°C and >39°C. Rectal temperatures in the control group were not affected by altering ambient temperature and 5-HT levels were normal. However, in comparison to control, 5-HT levels in the ≤39°C group were significantly reduced in all brain regions (str 40%, fc 23%, hipp 26%) while the 5-HT levels in the >39°C group (str 24%, fc 9%, hipp 17%) were significantly lower in all brain regions than in the ≤39°C group. Our results demonstrate that when a hyperthermic response occurs as a result of exposure to (+)Fen, 5-HT depletion is significantly greater than when there is no hyperthermic response.

1331 A STUDY OF GLIAL FIBRILLARY ACIDIC PROTEIN AS A NEUROBIOMARKER FOLLOWING A SINGLE ORAL EXPOSURE TO EITHER TOLUENE OR TRICHLOROETHYLENE IN RATS

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Toluene (TOL) and trichloroethylene (TCE) are two commonly found environmental pollutants that have been reported to be neurotoxic (The Toxicologist 14(1):1338 and 15(1):447). Many have reported that glial fibrillary acidic protein (GFAP), an astrocyte intermediate filament protein, to be a biochemical marker for astrogliosis, a neurotoxic response to nervous system damage (Ann NY Acad Sci 679:195, 1993). Groups of 10 male and 10 female Sprague-Dawley rats (42 days old) were used to compare GFAP levels with the degree of neuropathology following acute exposure to a single gavage dose of either TOL or TCE. The dosing regimen was: LOW (TOL 3.0 ml /kg or TCE 1.50 ml/kg); INTERMEDIATE (TOL 4.5 ml/kg or TCE 2.25 ml/kg); HIGH (TOL 6.0 ml/kg or TCE 3.00 ml/kg). Animals were sacrificed on day 14 and GFAP content, total protein, and histopathological examination was performed on the cortex (CTX), hippocampus (HP), brain stem (BS), and cerebellum (CRB). Compared to saline controls, TOL-exposed male rats had elevated GFAP in the HP, CRB, and BS (only at HIGH dose level), whereas female rats had elevated GFAP at HIGH dose level in the HP only. GFAP was not elevated in the CTX in either male or female rats. TCE-exposed rats had increased GFAP levels in the HP region only. These findings suggests that astrogliosis has a rapid onset after a neurotoxic insult. Furthermore, GFAP as a biomarker of this event can serve as a more sensitive indicator of early and subtle neurological damage than can be detected by histopathological examination. [Funded by ATSDR grant #U50/ATU398948-03].

1332 NEUROTOXICITY OF 1,3,5-TRINITROBENZENE (TNB): IMMUNOHISTOCHEMICAL STUDY

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TNB is a soil and water contaminant at certain military installations. We have reported encephalopathy in rats given TNB orally once daily for 10 days. The lesions consisted of bilateral symmetrical malacia in the cerebellar and vestibular nuclei and inferior colliculi. The pathogenesis and contribution of the blood brain barrier (BBB) were not defined in the initial report. Male F-344 rats (5/group) were killed after 4, 5, 6, 8, or 10 daily doses of TNB (71 mg/kg). Also 10 rats (5/gp) were allowed to recover for 10 or 30 days after receiving 10 doses. Integrity of the BBB was assessed immunohistochemically using staining for albumin. Serial sections were stained with H&E and for glial fibrillary acidic protein (GFAP). Rats killed after 4–8 doses had no lesions. In the 10 dose group, lesions were multifocal, often confluent foci of extravasated albumin in susceptible nuclei. Albumin was present in vascular walls, extracellular space, neurons and Purkinje cells. Immunoreactivity in neurons was granular representing pinocytic uptake or diffuse due to nerve cell injury with uncontrolled albumin leakage into the cytoplasm. Shrunken hypereosinophilic neurons were seen in areas of albumin extravasation with sponginess of the neuropil. There was no GFAP staining in the vacuolated foci, with hypertrophied astrocytes around the foci. After 10d of recovery, vacuolated foci were infiltrated by glial and gitter cells. Albumin immunoreactivity was present as granular debris and neuronal staining was decreased. A glia limitans around the resolving foci had thick bundles of GFAP positive astrocytic processes. In rats allowed a 30d recovery, immunoreactivity of the lesions were much less obvious. Leakage of albumin was not seen and GFAP was mild. These rats had necrotic neurons in the susceptible nuclei and loss of Purkinje cells. These observations document a relationship between increased vascular permeability (vasogenic edema) and neuronal damage in TNB encephalopathy.

1333 METABOLIC EFFECTS OF 3-NITROPROPIONIC ACID TREATMENT IN NEONATAL RAT BRAIN

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The mitochondrial toxin, 3-nitropropionic acid (3-NPA) causes deficits in energy metabolism by irreversible inhibition of succinate dehydrogenase of both the Kreb's cycle and complex II of oxidative phosphorylation. Impairment of energy metabolism may lead to secondary excitotoxicity and subsequently to free radical generation. We examined the acute effects of 3-NPA on succinate dehydrogenase (SDH) enzyme activity and the extent of lipid peroxidation (LPO) in the cerebral tissue. Male Sprague-Dawley rat pups (PND 5) were administered a single injection of 3-NPA at doses of 30 or 50 mg/kg, s.c. and returned to the dams. Three and six hours later, the pups were sacrificed and the brains were quickly removed. SDH activity was determined spectrophotometrically. Basal and induced (0.1mM ferrous sulfate) LPO levels were assessed in the cerebral homogenates by measuring the concentration of thiobarbituric acid reactive substances. Compared to control rats, the specific activity of SDH in the cerebral tissue was inhibited by 76 to 71% at the 30mg/kg dose, while there was 80 to 78% inhibition at the 50 mg/kg dose after 3 and 6 hr, respectively. A trend for elevated LPO was observed in the unstimulated tissues of 3-NPA treated rats. Compared to rats injected with 30 mg/kg 3-NPA, the products of induced LPO were significantly higher in rats receiving 50 mg/kg. Thus, the inhibition of SDH in neonatal rats by 3-NPA was associated with a higher sensitivity to lipid peroxidation.

1334 INDUCTION OF RAT BRAIN GST AND SERUM BILIRUBIN BY ACRYLAMIDE AND DIETHYLMALEATE

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Acrylamide (ACR), a neurotoxicant, depletes glutathione (GSH) and induces glutathione S-transferase (GST) in rat brain and liver. Depletion of GSH has

been shown to induce heme oxygenase-1 (HO-1/hsp32), a component of the heme metabolizing system, resulting in increased serum bilirubin, an endogenous neurotoxic product that may induce cerebellar lesions. This study investigated the relationship between GSH depletion, serum unconjugated bilirubin (SUB) levels and modulation of GST in rat brain and liver. Single administration of ACR (50 or 100 mg/kg; i.p.) to male Sprague-Dawley rats (250-300 grams; n = 3-6) decreased GSH in a dose-dependent manner in cerebellum (70 ± 7% and 49 ± 3% of control), cortex (72 ± 2% and 46 ± 2%) and liver (67 ± 7% and 40 ± 3%) respectively; brain and liver GST were unaffected 6 hours after the toxicant dose. ACR (50 mg/kg/day x 5 days) slightly decreased only cortical GSH, 24 hours after the last dose; GST was not affected in either brain or liver but there was an increase in SUB (153 ± 25%). ACR (50 mg/kg/day x 10 days) reduced cerebellar (86 ± 4%), cortical (72 ± 4%) and liver (62 ± 10%) GSH 6 hours after the last dose; GST was increased in cerebellum (129 ± 6%) and liver (152 ± 4%) but not in cortex. SUB was increased to 192 ± 15%. However, 24 hours after the last dose, GSH had returned to normal levels in cerebellum, cortex and liver, while GST remained elevated in cerebellum (143 ± 7%) and liver (128 ± 2%) but not in cortex. The GSH depletor diethylmaleate (DEM-50 mg/kg/day x 10 days) reduced cerebellar (77 ± 7%), cortical (61 ± 7%) and liver (51 ± 5%) GSH 6 hours after the last dose, while increasing GST in cerebellum (136 ± 5%) and liver (134 ± 7%), but not in cortex. SUB levels were increased by 188 ± 14%. These results indicate that depletion of GSH by ACR and DEM induces GST in rat cerebellum and liver; this induction of GST in rat cerebellum may reflect the effects of GSH depletion on the heme metabolizing system that may also play an initiating role in the CNS neurotoxicity of ACR. (Supported by NIH ES-06356, ES-05022, ES-05955).

1335 TIME-COURSE OF LEAD-INDUCED CHANGES IN D1, D2 AND DAUC BINDING LEVELS

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Prior studies have frequently relied on single exposure concentrations and durations to define lead (Pb)-induced changes in dopamine (DA) binding levels. This study tracked the effects in rats of chronic postweaning exposure to levels of 0, 50 and 150 ppm Pb acetate on dopamine D1, D2 and DAUC (dopamine uptake carrier) binding levels assessed by autoradiography after 2 weeks, 6 mo and 10 mo of exposure. Pb exposure did not affect binding in striatum (STR) at any time point while decreasing binding, particularly of D2 and DAUC sites, in nucleus accumbens (NAC). Chronic intermittent i.p. administration of the D1 agonist SKF82958 or the DA agonist apomorphine decreased D1 and D2 binding in the 0 ppm group in NAC at 6 mo and in STR and NAC at 10 mo. These same treatments increased D1 and D2 binding levels in both STR and NAC of the 150 ppm group at 6 mo but reduced D1 and D2 binding of 50 and 150 ppm groups after 10 mo. DA agonist treatments did not systematically alter DAUC binding in the 0 ppm group at 6 or 10 mo, but SKF82958 decreased DAUC binding in the 50 and 150 ppm groups at 10 mo. These data suggest a preferential vulnerability of NAC and of D2 and DAUC sites to Pb exposure. Chronic DA agonist treatments induce differential effects in Pb-treated rats where DA systems are already altered by Pb exposure per se. ES05017, ES05903 and ES01247.

1336 EXCITOTOXIC LESIONS OF THE RAT MAMMILLARY BODY IN A THIAMINE DEFICIENCY MODEL OF WERNICKE-KORSAKOFF'S SYNDROME

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Up to 12.5% of alcoholics suffer from Wernicke-Korsakoff's Syndrome (WKS), a disease characterized by cognitive and memory impairments, and lesions of the thalamus and mamillary body. This disease can be modeled in rats by an acute bout of pyrithiamine-induced thiamine deficiency (PTD). Evidence from this model suggests that thalamic lesions involve glutamate-NMDA receptor mediated excitotoxicity (Langlais, *Metabolic Brain Disease* 10:31–44, 1995). The present study examined the role of NMDA receptors in PTD induced lesions of the medial nuclei of the mammillary body. Male Sprague-Dawley rats were treated daily with 0.25 mg/kg ip pyrithiamine, and fed a thiamine deficient diet. At the onset of symptoms, the animals were assigned to one of three treatment groups: 3.0 mg/kg/day MK-801, 1.5 mg/kg/day MK-801, and saline. Treatment continued for 12 hours following reversal with a large dose of thiamine (100 mg/kg ip). Five days following reversal, brains were removed, fixed, and examined along with archival slides of pair-fed and ad lib control animals. Results indicate that both doses of MK-801, a noncompetitive NMDA receptor antagonist, had a significant ($p<.05$) protective effect on neuronal loss and severity of gliosis, with greatest protection in the ventral area. There was no significant loss of neurons or increase in gliosis in the ML of any of the treatment groups. These results indicate that NMDA glutamate excitotoxicity is significantly involved in PTD neuronal loss and gliosis in the MM, while the ML is not significantly affected by thiamine deficiency. These findings suggest that glutamate antagonists may prove effective in preventing pathological and behavioral disturbances in human WKS patients. Supported by NIH grant NS29481 and VA Merit Program.

1337 SUBCHRONIC TOXICITY ASSESSMENT OF LY315535, A 5-HT1a AGONIST/MUSCARINIC ANTAGONIST IN FISCHER 344 RATS

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Daily oral doses of LY315535, a potent 5-HT1a agonist, with mild antimuscarinic activity, were administered to Fischer 344 rats for one month. Mortality occurred in rats given 24 mg/kg or 8 mg/kg. Deaths were attributed to convulsions which were observed throughout the treatment period, as was chromodacryorrhea. Other clinical signs, components of the serotoninergic behavioral syndrome, and decreased heart rate were observed from Day 1 to Day 5. These transient effects have been reported in the literature as direct central effects of 5-HT1a agonists (Mir and Fozard, 1990; Larsson, et al., 1990). Decreases in body weight associated with decreases in food consumption were observed during week 1, followed by a progressive recovery. Increased neutrophil and monocyte counts, increased adrenal weight, thymic involution, and liver changes (leukocytosis, activation of Kupffer cells, and single cell necrosis) accompanied by increased plasma ALT, AST, and ALP values were observed. These findings may be the result of an increase in endogenous corticoid level. Corticosterone plasma levels were assayed in separate groups of rats treated under the same conditions. A dose-related increase of corticosterone level occurred on Day 1 in all treated groups. This effect decreased with time, such that on Day 27, corticosterone plasma levels were no longer affected by the treatment. These observations are probably not the result of changes in metabolism, but are consistent with the pharmacological properties of 5-HT1a agonists to elicit the release of corticosteroids, known to be markedly attenuated after repeated administration (Kelder and Ross, 1992). Indeed, plasma levels of the parent compound were generally unaffected by the duration of treatment while exposure to 2 pharmacologically active metabolites was increased between Days 1 and 28.

1338 ANTIDOTAL EFFECT OF DIHYDROXYACETONE ON CYANIDE INTOXICATION

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Cyanide (CN) intoxication in mice can be effectively antagonized by dihydroxyacetone (DHA), particularly if administered in combination with another CN antidote, sodium thiosulfate. CN-Induced convulsion was also prevented by DHA treatment, either alone or in combination with thiosulfate. Injection (ip) of DHA (2 g/kg) 3 min after or 10 min before CN (sc) increased LD_{50} values of CN (7.8 mg/kg) by a factor of 2.1 and 3.0, respectively. Posttreatment with the combination of DHA with thiosulfate (1 g/kg) increased the LD_{50} of CN by a factor of 2.4, while pretreatment with this combination increased the LD_{50} to 83 mg/kg. Posttreatment with α-ketoglutarate (2 g/kg), but not pyruvate, increased the LD_{50} for CN by a factor of 1.6, whereas the currently used nitrite-thiosulfate combination antidote was less effective. Pretreatment with DHA also prevented the inhibition of brain, heart, and liver cytochrome oxidase by CN, and posttreatment with DHA accelerated the recovery of cytochrome oxidase activity especially in brain and heart. The antagonizing mechanism of DHA may involve cyanohydrin formation as DHA rapidly restored hepatocyte respiration blocked by CN (Niknahad *et al.*, 1994, *Toxicol. Appl. Pharmacol.*, 128, 271–279). DHA is a physiologic component of the cell which supplies ATP via glycolysis. It can make a safe and effective antidote for CN, especially in cases of CN intoxication resulting from fire smoke inhalation in which a combination of CN and carbon monoxide is present, and administration of the methemoglobin inducer sodium nitrite is contraindicated, because carbon monoxide complexes hemoglobin.

1339 METAL CONCENTRATIONS AND HISTOPATHOLOGY OF BRAIN AFTER SUBCHRONIC ZINC OXIDE

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The concentrations of three metals and histopathology were used as part of an initial screen for neurotoxicity of zinc oxide. Male Sprague-Dawley rats were feed *ad lib.* diets containing 18.6, 114.9, 339.6, 981.5, 3228, or 6447.9 μg of zinc/g of diet for ninety days. Zinc citrate contributed 18.6 μg of zinc/g to each diet. Zinc oxide was used as the source of zinc in all diets containing more than 18.6 μg of zinc/g of diet. The brain was bisected along the midline. Half of each brain was used for metal analysis, and the other half for histopathologic analysis. All brain sections were stained with the Holmes' silver stain. Analysis of brain sections suggest changes in the cerebellum of rats consuming the highest dietary zinc. The metal concentrations were measured by an Inductively Coupled Plasma Atomic Emission Spectrometer with an ultrasonic nebulizer. Zinc brain concentrations were significantly higher in the rats consuming the diet containing 6447.9 μg of zinc/g, relative to all rats on other diets. The copper brain concentrations were not significantly different from controls. The iron brain concentrations were higher relative to controls in only the rats consuming a diet with 114.9 μg/g. In summary, dietary zinc can significantly influence the brain metal concentration. (Partial support by ATSDR grant U50/ATU398948--03)

1340 CALCIUM- AND ZINC- DEPENDENT PROTEASES PARTICIPATE IN HIPPOCAMPAL PLASTICITY FOLLOWING TRIMETHYLTIN ADMINISTRATION

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Loss and subsequent reexpression of specific cytoskeletal proteins and adhesion molecules are crucial events in the 'plastic' response of neural cells to injury. Of particular relevance to neuronal remodelling is the ability of toxicants to perturb divalent metal homeostasis at synaptic sites. Activation of calcium- and zinc- dependent proteases represents a possible mechanism by which rapid changes in synaptic structure may be effected. The neurotoxicant trimethyltin (TMT), known to alter cellular calcium and zinc levels, causes loss of the synapse-specific neural cell adhesion molecule NCAM180 and dendritic cytoskeletal elements in mouse hippocampus within 4-12 hr, prior to observation of neuronal death. The current study examined the role of proteases in TMT-induced cytoskeletal and NCAM loss. Mice were given a single i.p. injection of either 2.0 or 3.0 mg/kg TMT, doses associated with mild and extensive neuronal death, respectively, and killed at various time-points. Hippocampi were isolated, homogenized and incubated for 2 hr at 37°C with the endogenous substrate azocasein and 5mM calcium or zinc. Proteolytic cleavage of azocasein was determined spectrophotometrically and standardized to protein values. Both calcium- and zinc- dependent proteolysis were significantly elevated relative to control, with maximal elevations 6 hr after treatment. Calcium-dependent proteolytic activity was 48% greater than controls, while zinc-dependent activity was 67% higher. Proteolytic activities of TMT-treated mice returned to control values 24-48 hr after treatment, times coincident with the reappearance of cytoskeletal markers and NCAM180 confirmed by Western blotting. Activation of proteolytic enzymes due to TMT-induced disruption in divalent cation homeostasis may be a means by which normal adhesion between cells is disrupted, facilitating cellular plasticity and tissue remodelling. (Supported by ES-04976, ES-06103 and an USEPA Graduate Fellowship [PMD])

1341 THE TEMPORAL INDUCTION OF STRESS PROTEINS IN RAT HIPPOCAMPUS FOLLOWING KAINIC ACID ADMINISTRATION

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Kainic acid (KA) is an exogenous glutamic acid analogue derived from Japanese seedweed, *Digenea simplex*. It is a potent neurotoxicant that has been shown in the mammalian nervous system to cause over stimulation of neurons. This overt neuronal excitation contributes to cellular insult and perhaps neurodegenerative disease. Studies have shown that excitotoxicity plays a major role in the cellular stress response in the CNS. Although the cellular mechanism responsible for neuronal injury is not clearly elucidated, it is evident that the activation of amino acid excitatory receptors by KA leads to numerous cellular alterations including the induction of stress proteins. To better understand the relationship between the cellular stress response and the excitotoxicity of KA, we examined its ability to induce hsp72 and hsp32 in the rat hippocampus. Male Long-Evans (LE) rats were injected subcutaneously (s.c.) with 10 mg/kg of KA (pH 7.4). At specific time points (0 – 72hr), animals were sacrificed by rapid decapitation and the hippocampus was quickly dissected and stored at -80°C until time of analysis. The expression of hsp72 and hsp32 at each time point was analyzed electrophoretically followed by immunoblotting. In addition, the dose-response effect of KA-induced stress proteins was also investigated. Male LE rats were injected subcutaneously (s.c.) with varying doses of KA (0.5 – 10 mg/kg, pH 7.4). Twenty-four hours later rats were sacrificed and the hippocampus was quickly dissected, each dose was analyzed as described above. The data indicates that over a 72 hr period, differences were observed between the time dependent induction of hsp32 and hsp72.

1342 DEVELOPMENTAL EXPOSURE TO LEAD (Pb) ALTERS NMDA RECEPTOR MODULATION BY GLYCINE AND ZINC IN THE RAT BRAIN

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We have previously shown that developmental exposure to Pb results in alterations in NMDA receptor levels in the rat brain (Jett & Guilarte, *Neuro-Toxicol.* 16:7–18,1995). In the present study, we examined the effects of the NMDA receptor co-agonists glutamate and glycine, and the inhibitory allosteric modulator zinc on [^{3}H]-MK-801 binding to cortical membranes from postnatal day (PN) 14, 28, and 56 rats developmentally exposed to 75, 250, or 750 ppm Pb-acetate in the diet. The results indicate no significant changes in the potency (EC_{50}) or efficacy (maximal enhancement) of glutamate stimulation of [^{3}H]-MK-801 binding to cerebral cortex preparations. On the other hand, there was a significant increase in the glycine EC_{50} (reduced potency) with no change in maximal enhancement of [^{3}H]-MK-801 binding to cortical membranes from PN14 and PN28 rats exposed to the highest level of Pb. As previously described, we also measured high and low affinity binding sites for zinc on the NMDA receptor complex (Guilarte et al., *Neuro-Toxicol.* 16:63–72,1995). Pb exposure resulted in a significant increase in the zinc IC_{50} for the low affinity site in cortical membranes from all Pb-treated groups relative to controls at PN 28 and 56. No significant effects on the number of low affinity sites or in any parameter for the high affinity site were found. These findings suggest that developmental exposure to Pb alters the glycine and zinc modulation of the NMDA receptor complex in the rat cerebral cortex. [Supported by grant ES06189 and ES03819]

1343 CHANGES OF DOPAMINE TRANSMISSION AND DOPAMINE RECEPTOR BINDING PARAMETERS BY MK-801 IN MORPHINE DEPENDENT RATS

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In order to investigate the effects of chronic treatment of MK-801, noncompetitive N-Methyl-D-Aspartate receptor antagonist, on the morphine physical dependence and dopaminergic regulation in rat brain, we measured the changes of the development of physical dependence including behaviors and those in dopamine transmission and receptor binding characteristics. Pretreatment(ip) of MK-801 every 30 min before administration of morphine(sc) twice a day for 7days inhibited the naloxone precipitated withdrawal symptoms in Sprague-Dawley male rats. During the withdrawal period, extracellular concentration of dopamine in caudate putamen assayed by *in vivo* microdialysis was decreased and the contents of its metabolites, DOPAC and HVA, were increased. Coadministration of MK-801 with morphine attenuated the decreasing rate of dopamine and increasing extents of its metabolite concentrations in caudate putamen of morphine dependent rats, finally to the normal levels. Binding parameters of [^{3}H]SCH23390 and [^{3}H] Spiperone in striatal membrane preparations were not significantly different in control and morphine dependent rats. However, MK-801 pretreatment during chronic administration of morphine increased the Bmax value for [^{3}H]SCH23390 in D1 receptors comparing to that of morphine dependent rats, while there were no differences in Kd and Bmax for [^{3}H]Spiperone in D2 receptors. Those results suggest that MK-801 affect the development of opioid dependence and dopamine transmission with up-regulation of D1 receptor.

1344 ASTROCYTES AND MICROGLIA REACTIVITY IN RAT HIPPOCAMPUS FOLLOWING NITRO-L-ARGININE METHYL ESTER (NAME) AND TRIMETHYLTIN (TMT)

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To test the hypothesis that the L-arginine-nitric oxide pathway in the brain plays a role in chemically-induced neuropathology of the hippocampus, we examined the ability of the nitric oxide synthase inhibitor NAME to modulate TMT neurotoxicity. Adult male Long-Evans rats were injected with NAME (50 mg/kg) daily for 4 days; on the final day of treatment, animals received a single sc injection of 4 mg/kg TMT. After 4 and 30 days, morphological alterations in hippocampal astrocytes, microglia and neurons were examined with light microscopy using GFAP staining, lectin-binding, and H&E staining as respective cellular markers. Consistent with previous studies, TMT produced a mild neuronal necrosis in dentate granule cells by 4 days, which progressed to extensive neuronal loss and astrogliosis in the CA3–4 region by 30 days post dosing. The density of astrocytic processes was increased within 4 days after administration of NAME. By 30 days post NAME, a mild neuronal necrosis of the CA1-2 pyramidal cells and dentate granule cells was evident. Co-administration of NAME and TMT did not alter the overall pattern of TMT-induced neuropathology; however, astrocytes displayed short, thick processes. There was also an increased number of ameboid microglia. Reactive neuroglial was associated with increased expression of mRNA for the pro-inflammatory cytokines IL-1α, IL-6 and TNFα, as determined by RT-PCR. These data demonstrate that neuroglia response is regulated by nitic oxide and that TMT neuropathology may not be directly associated with an excito-toxic response. (WEM supported by EPA #CT901915).

1345 IBOGAINE: NEUROHISTOLOGICAL STUDIES IN MICE AND RATS

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The psychoactive indole alkaloid ibogaine (IBO) is being considered as a treatment for drug addiction, but it's side-effects require study. Single doses of IBO (30-100 mg/kg, ip) caused tremors and necrosis of cerebellar Purkinje cells in rats (O'Hearn and Molliver, 1993). We administered IBO (100 mg/kg IP) or saline to adult rats and mice and perfused them one hr, 24 hrs, or 6 days later. Cerebellar sections were stained for degeneration with silver and a novel fluorescence method. Patches of necrotic Purkinje neurons were present in the cerebellar vermis of dosed rats, but not mice. Intense c-fos immunoreactivity was seen in neocortical cells one hr after IBO (but not after saline or at any other survival interval). Layer 2 of neocortex was selectively effected in mice, while rats had an even activation throughout the cortex. Our results confirm the previously reported neurotoxicity of IBO in rats and show pharmacological sites of action of IBO in the forebrain consistent with its psychoactivity. We hypothesize that neuroanatomical differences in cortical projections to the cerebellum may underly the sparing of Purkinje cells in the mouse.

1346 NALOXONE-PRECIPITATED κ-OPIOID RECEPTOR AGONIST WITHDRAWAL INCREASES GLUTAMATE LEVELS IN RAT LOCUS COERULEUS

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The extracellular fluid (ECF) levels of excitatory amino acids (EAA) (glutamate, Glu and aspartate, Asp) in the locus coeruleus (LC) of butorphanol ($\mu/\delta/\kappa$-mixed opioid receptor agonist)-and U-69,593 (a selective κ-opioid receptor agonist)-dependent rats precipitated by naloxone were investigated by *in vivo* microdialysis. The behavioral signs of naloxone-precipitated withdrawal were also observed. Increases in ECF levels of Glu and Asp were noted after naloxone (48 nmol/5 μl, LC) precipitated withdrawal in the rats which had been intracerebroventricularly infused with butorphanol (26 nmol/1 μl/hr) or U-69,593 (26 nmol/1 μl/hr) for 3 days. Furthermore, behavioral evidence of withdrawal (teeth-chattering, wet-dog shakes, etc) was detected following naloxone challenge in the butorphanol- and U-69,593- infused rats, but not in saline-infused controls. These results provide direct evidence to support that the role of EAA within the LC is involved in butorphanol- and U-69,593-withdrawal. It is further indicated that there is a significant interaction between EAA neurotransmission and a κ-opioid receptor in the LC which mediates butorphanol- or U-69,593-induced withdrawal. (Supported by NIDA DA-05828)

1347 POST-NATAL DEVELOPMENT OF P-GLYCOPROTEIN, A COMPONENT OF THE BLOOD-BRAIN AND INTESTINAL BARRIERS, IN RATS

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P-glycoprotein (p-gp), a transmembrane ATP-dependent transporter, is associated with the multiple-drug resistance phenotype (*mdr*). This protein functions as a transporter of a variety of xenobiotics decreasing their intracellular concentrations and has also been implicated as an important component of the blood-brain barrier (BBB), preventing entry of compounds into the CNS. In addition, p-gp in the intestinal epithelium reduces absorption of compounds into the systemic circulation. The avermectins, a group of complex, macrocyclic natural products used as parasiticides and insecticides, have been shown to be substrates for p-gp. Since neonatal rats are significantly more sensitive than adults to the neurotoxicity of the avermectins, we examined the post-natal development of p-gp in rats using a mouse monoclonal antibody to the protein (C219) and standard immunohistochemistry methods. The results confirm that p-gp in brain capillary endothelia is incomplete at birth in rats and increases with age, maturing about 5 weeks postpartum. In the intestine, no p-gp was evident until about 8 days after birth with levels also maturing at about 5 weeks. These results agree with previous methods showing that the BBB is incompletely developed at birth in rodents and correlate with the post-natal sensitivity of rats to the avermectins. Additionally, the delay in the development of an intestinal barrier to absorption may also contribute to neonatal sensitivity via oral exposure to compounds. In view of the reported maturity at birth of the BBB in primates, including humans, neonatal rats may not be predictive of the sensitivity of other species to neurotoxic compounds which are substrates for p-gp.

1348 REGIONALLY DIFFERENTIAL CHANGES OF CYCLIC GMP LEVELS IN THE BRAINS OF MICE AND RATS UPON 1,1,1-TRICHLOROETHANE EXPOSURE

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The potential impact of differential regional brain distribution of 1,1,1-trichloroethane (TRI) in rodents on the regionally definable neurotoxicology was evaluated by examining the effects of TRI inhalation on the cyclic GMP levels in discrete brain regions of rats and mice. Both species were exposed to 5000 ppm TRI by inhalation for either 40 or 100 min. Following focused microwave irradiation on the animal heads, the animal brains were removed and dissected into medulla oblongata (MO), cerebellum (CB), hypothalamus (HY), hippocampus (HC), striatum (ST), midbrain (MB), and cortex (CX) for rats, and MO, CB, and CX for mice. The cyclic GMP level in each sample were measured by radioimmunoassay. In control animals, difference in cGMP levels were found between the regions, with cerebellum showing significantly higher cGMP than other regions in both rats and mice. After 40 min of TRI exposure, significant decreases of cGMP were observed with mouse in both MO and CB (52% and 58% from control, respectively). Similar changes were also shown in rat CB (55%) and CX (27%) after 40 min of TRI inhalation. Regional changes in cGMP levels are not directly correlated with the known ranking order of TRI uptake and accumulation in those brain regions. Between the two species, it was found that the rats had higher cGMP levels than mice in both control and exposure groups for all three regions that were measured in both species. Although a regionally differential effect was seen in the changes in cGMP levels in rodent brains following TRI exposure, it seems that the differential distribution of TRI in the corresponding brain areas is not solely responsible for that. (Supported by AFOSR 910356)

1349 DIETHYLDITHIOCARBAMATE (DDC): A PROMOTER OF DOPAMINERGIC TOXICITY

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DDC is one of a family of widely used agricultural, industrial and pharmaceutical chemicals (the dithiocarbamates, DTCs). Exposure of humans to DTCs has been associated with neurological deficits including the development of extrapyramidal movement disorders characterized by hypokinesia and tremor and responsive to L-DOPA therapy, a clinical syndrome of parkinsonism

strongly suggestive of disturbances in nigrostriatal dopamine (DA). Although experimental studies with DDC have not demonstrated any direct DA toxicity for this compound, DDC has been shown to enhance the neurotransmitter-depleting and other toxic effects of the DA neurotoxin MPTP. The purpose of the present study was to investigate the interaction of DDC with other known and suspected DA toxins.

DDC (400 mg/kg) or saline was administered to groups of 8 week old male C57BL/6 mice. One-half hour later animals were given either MPTP, methamphetamine or L-DOPA (administered as the L-DOPA methyl ester): benserazide. Appropriate groups received either DDC + saline, saline + saline or saline + drug and served as controls. Animals were sacrificed 5–21 days later and striatal DA assayed by HPLC/EC.

Although DDC alone had no effect on striatal DA, combined treatment significantly enhanced the effects of MPTP and methamphetamine. More remarkably, DDC appeared to confer DA depleting ability on L-DOPA, a compound that is without such effects when administered alone. The ability of DDC, a ubiquitous environmental pollutant to act in combination with L-DOPA, the endogenous precursor to dopamine provides a new and potentially interesting model to investigate mechanisms of nigrostriatal neurodegeneration in conditions such as Parkinson's disease. These findings also lend credence to the possibility that one or more environmental compounds may contribute to the etiology of this disorder.1

1350 IDENTIFICATION AND PURIFICATION OF BRAIN-FABP AND HEART-FABP FROM MOUSE BRAIN

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The exofacial leaflet of brain synaptic plasma membrane (SPM) is significantly more fluid than the cytofacial leaflet, and contains 13% of SPM cholesterol, substantially less than in the cytofacial leaflet (87%) of young mice. Both increasing age and chronic alcohol consumption decreased the transbilayer fluidity gradient and increased cholesterol two fold in the exofacial leaflet. However, total SPM cholesterol did not change. Cholesterol redistribution may account for the decrease in transbilayer fluidity gradient observed with aging and alcohol consumption. Cytosolic lipid binding proteins such as fatty acid binding proteins (FABPs) can bind hydrophobic ligand such as fatty acid, cholesterol and may control lipid transport and metabolism, thereby contributing to cholesterol redistribution in brain SPM. Two native proteins, B-FABP and H-FABP were isolated from Swiss Webster mouse brains using conventional chromatographic methods with yield 1.4% and 22%, and purification factor 950 and 9,600 respectively. Native B-FABP has a molecular weight 14.9KDa, pI 4.5. Native H-FABP has a molecular weight 14.8KDa, pI4.9. These data show for the first time the coexistence of two native FABPs in brain. The amino acid compositions have been compared with recombinant B-FABP and H-FABP. Their function in lipid trafficking and distribution is under investigation.

1351 EFFECT OF ACUTE THALLIUM ADMINISTRATION ON GABA LEVELS OF RAT BRAIN HYPOTHALAMUS

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Thallium is a very toxic metal, in Mexico and other countries, intoxication with this metal is still considered a health problem. Its clinical manifestations include central nervous system alterations such as ataxia, weakness, somnolence, hyperflexia, convulsions and coma. In previous studies we reported preferential accumulation of thallium in hypothalamus. GABA is the most important inhibitor neurotransmitter in brain and its increased levels been associated to coma. Control rats received saline solution, i.p. and treated rats a thallium acetate dose (16 mg/kg, i.p). Either 1 or 7 days after treatment animals were killed and dissected out their hypothalamii samples were homogenized, centrifuged and supernatants analyzed by HPLC with a fluorescent detector. We found a 29% increase of GABA levels in treated rats at 24 hr while at 7 days, rats didn't show any change. Data suggest an acute alteration of hypothalamic GABA. CONACYT GRANT 0935P-M9506.

1352 INTRAPERITONEAL KAINIC ACID PRODUCED INCREASE IN SEROTONIN TURNOVER RATE IN RAT EPILEPTOGENIC BRAIN REGIONS

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Kainic acid (KA) induces severe convulsions and neurotoxicity in rats. Research with this model has revealed marked changes in noradrenaline (NA), dopamine (DA) and serotonin (5-HT) metabolites. In the present study KA was injected (i.p. 10 mg/kg) to rats, and serotonin turnover rate was assessed in 5 brain regions: frontal cortex (C), hippocampus (H), striatum (S), amygdala (A) and midbrain (M). We tested 4 different groups: 1) Rats injected with saline solution (SS) + SS, 2) KA + SS 3) Pargyline (P; 75 mg/kg) + SS and 4) KA + P. P was used to estimate 5-HT turnover rate. KA was injected 2 and 24 hrs prior to a second injection with P. One hour later, all animals were killed to analize 5-HIAA dissapearence. We observed an increased 5-HT turnover rate at 2 hr in M(19%), S (35%) and A (38%). At 24 hr after KA administration results were M (19%), S (16%), C (79%), A (107%) and H (31%). Results indicate that 5-HT turnover rate was higher at 24 hrs than at 2 hr in brain regions mainly in A, C and H.

1353 EFFECT OF ETHANOL (ETOH) PRETREATMENT ON NEUROCHEMICAL CHANGES ASSOCIATED WITH METHAMPHETAMINE (METH)-INDUCED NEUROTOXICITY

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Neurotoxicity of **METH** has been attributed, in part, to its hyperthermic effect which can be fatal. Previous findings in our lab show that pretreatment with **ETOH** provided complete protection against this hyperthermic effect without affecting **METH**-induced stereotype behavior (**STB**). The present study was designed to examine the neurochemical changes associated with repeated exposure to **METH** alone and **METH** following **ETOH** pretreatment. Male Sprague-Dawley rats (+ 350 grams) were treated i.p. every 2 hrs with: **METH** (5 mg/kg), **ETOH** (2 g/kg), **ETOH** followed 15 minutes later by **METH** or saline. Animals were sacrificed at 2 hr intervals following treatment. Various brain regions were dissected and the neurotransmitter levels were determined by **HPLC**. The results show that **METH** causes significant decrease of **DA** in the striatum and nucleus accumbens, after 2 and 8 hours, respectively, as compared to controls. This is consistent with behavior data previously reported. **DA** levels in olfactory bulb were significantly increased in the three treated groups at all time periods compared to controls. No significant changes in **DA** levels were found in the hypothalamus in response to **METH**, but there was a slight increase in **DA** for both groups receiving **ETOH**. **ETOH**, either alone or with **METH**, caused significant increases in **NE** levels in the hypothalamus and thalamus after 1, 2 or 3 injections compared to controls. These results suggest that **METH**-induced **STB** and hyperthermia are related to changes in **DA** and/or **NE** or other monoamines in specific brain regions. (Supported by CDC/ATSDR/ **MHPF** Grant #U50/CCU 304522–04, NIH/MBRS grant GM 08111 and NIH/RCMI RR 03020)

1354 IDENTIFICATION OF RESPIRATORY ALLERGENS: INTRODUCTION

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A variety of chemicals and proteins can sensitize the respiratory tract. Among these are materials of industrial importance including certain diisocyanates, acid anhydrides, reactive dyes, and enzymes. Currently, there exist no widely accepted or well validated methods for the prospective identification of respiratory allergens. Most progress has been made with guinea pig methods where sensitizing potential is usually measured as a function of changes in pulmonary function induced following sensitization and challenge. However, these methods are usually prohibitively expensive, particularly for screening purposes. A number of alternative approaches are under consideration and will be the subject of this workshop. There will be short reviews of the nature of the health problems associated with occupational respiratory sensitization, chemical structure activity analysis as a tool for detecting pulmonary allergens, approaches used to test for respiratory allergens in guinea pigs, and alternative approaches using mice. Finally, regulatory issues and needs in

this area will be reviewed. There will then be open discussion of the relative merits and limitations of the approaches described, their appropriateness for various industrial and regulatory situations, and research and development needs. This abstract does not reflect EPA policy.

1355 ENTRY OF WOMEN INTO EARLY CLINICAL TRIALS: UPDATE ON PRECLINICAL AND CLINICAL ISSUES

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Recently, the FDA has removed restrictions on enrollment of women of childbearing potential into clinical trials for development of drugs and biologics. This action will permit evaluation of possible gender differences with respect to toxicity and efficacy of new agents. In traditional drug development, FDA required that women be enrolled in clinical trials after the submission of nonclinical reproductive and developmental toxicology studies, generally performed in parallel with Phase 2 clinical trials. However, for life-threatening indications such as AIDS, because of the different risk/benefit profile for potential therapies, FDA has permitted early enrollment of women, before submission of any reproductive studies. This Workshop will include timely presentations of FDA and European regulatory perspectives on timing of enrollment of women into early clinical trials, and the preclinical data that pharmaceutical manufacturers are developing to support these studies. Additional speakers will discuss the impetus within the women's community to enroll women earlier in clinical trials, the physician viewpoint on this initiative, and potential institutional and ethical concerns.

1356 REGULATION OF CELL PROLIFERATION AND APOPTOSIS IN CANCER: AN INTRODUCTION

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Abnormal regulation of the life cycle of cells is a major feature of neoplasia. Most malignancies are characterized by abnormalities of cell proliferation, typically a capacity for persistent and excessive proliferation, often at accelerated rates. In addition, abnormalities of cell death, often manifested as cell immortality, are another common feature of neoplasia. This symposium will provide a broad perspective on these abnormalities in preneoplastic and neoplastic cells and it will delve into mechanisms by which they contribute to cancer. In broad perspective these alterations of cell proliferation and apoptosis will be considered for hepatocarcinogenesis. This will span the issues of carcinogen dose, risk assessment, and the alterations of cell cycle regulatory proteins within emerging liver neoplasms. The features and mechanisms of cell death will be considered and morphological manifestations will be linked to molecular mechanisms. This will be further explored with regard to alterations of levels of ionized cellular calcium and the induction of newly identified genetic pathways leading to induced cell death. Mechanisms underlying these phenomena will be explored with regard to the failure of the G_1/S and G_2/M cell proliferation checkpoints during carcinogenesis. The abnormalities of these protective mechanisms will be linked to the progressing genetic instability that develops early in carcinogenesis and potentiates further genetic changes, abnormalities of cell senescence and reexpression of telomerase. The vulnerability of cells to chemical carcinogens when they are treated in the early part of the S phase will also be considered. Mechanisms for this vulnerability will be explored with regard to the vulnerability of replicating DNA to carcinogen adduction. In addition, studies will be considered which involve efforts to identify sequences and genetic loci in genomic DNA that are replicated in early S phase and may be critical DNA targets for carcinogenesis.

1357 GROWTH REGULATION IN HEPATOCARCINOGENESIS

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Altered growth and dysregulation of the cell cycle are a key feature of neoplasia. Survival and growth of initiated cells, preneoplastic lesions, and tumors are dependent on the rates of both cell proliferation and cell death. A large database is required to establish the associations between tumorigenicity and the extent, duration, and nature of chemically induced growth responses within both nonlesion and lesion cell populations. Examples of alterations in hepatic cell proliferation and apoptosis induced by different doses and types of chemical treatments will be presented. Cancer risk models require the quantitation and kinetics of chemical effects on the growth and loss of specific cell populations throughout the carcinogenic process. Alterations in growth factors, receptors, and cell cycle genes are critical for the altered growth regulation observed in hepatocarcinogenesis. Cyclin D. myc, and insulin growth factor II exhibited altered gene expression in liver tumors from mice. Transforming growth factor beta and the mannose-6-phosphate receptor were found to be important in regulating hepatic focal clonal growth. We are hypothesizing that cell cycle protein complexes or checkpoint controls represent the potential molecular targets for hepatocarcinogens. Cyclin-CDK complexes have been identified and characterized in mouse hepatocyte cell lines and in B6C3F1 isolated mouse hepatocytes. Attenuation of checkpoint responses was found to be associated with immortalization. Further delineation of chemical interactions with key cell cycle proteins should greatly improve our understanding of hepatocyte growth control mechanisms. As these relationships become clearer, investigations of carcinogenic mechanisms, assays for growth alterations, selection of doses for cancer bioassays, and formulation of risk assessment models will be improved.

1358 CELL DEATH AND CARCINOGENESIS

B F Trump. *Department of Pathology, University of Maryland School of Medicine, Baltimore, MD*

The process of carcinogenesis involves a balance between cell proliferation and cell death. It is our hypothesis that all complete chemical carcinogens, at effective doses, induce cell death. All effective cancer chemotherapeutic or genetic agents induce cell death or terminal differentiation in the cells of the neoplasm. It follows from this that an understanding of cell death and its consequences is fundamental to the understanding and control of neoplasia. Cell death is defined as the point where a cell cannot maintain internal homeostasis and reaches physiochemical equilibrium with its environment. The changes that occur following cell death are termed necrosis and two types occur: apoptotic and oncotic necrosis. The changes that occur prior to cell death are termed prelethal with two principal morphological patterns existing: apoptosis and oncosis. In apoptosis, the cells shrink, chromatin condenses, DNA fragments, and cytoplasmic blebs occur. In oncosis, the cells swell with dilatation of the cytosol and the endoplasmic reticulum, mitochondrial swelling, and chromatin clumping. The mechanisms of cell killing involve calcium signalling and activation of proteases, endonucleases, and phospholipases. Recently, a series of pro (p53, myc, waf 1, ced-3, ICE family, apopain), and anti (ced-9, bcl-2) cell death genes have been identified. The mechanisms of actions of pro-death genes have yet to be established, but protease and antioxidant activities appear to be prominent. However, it may be that the ultimate activity involves calcium mediated phospholipases, resulting in destruction of plasmalemmal integrity. In summary, cell injury and cell death are fundamental to the prevention, progression and treatment of neoplasia. [Trump, B.F. and Berezesky, I.K.: Calcium-mediated cell injury and cell death. FASEB J. 9: 219–228, 1995.] Supported by NIH grant DK15440.

1359 CELL CYCLE CHECKPOINTS, GENETIC INSTABILITY, AND INITIATION OF CARCINOGENESIS

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Carcinogens may initiate the process of carcinogenesis by causing mutation in cell cycle checkpoint genes that brake movement through the cell cycle. Checkpoints serve as both dependency controls and DNA damage-response elements. Thus, p53 has been shown to participate both in the G_1 checkpoint response to DNa damage and in the mitotic-dependence checkpoint that couples initiation of DNA synthesis to completion of mitosis. Human foreskin fibroblasts were transformed with the HPV E6 oncogene to inactivate p53. Cellular aging was induced by passaging. Soon after transformation and inactivation of G_1 checkpoint function, the G_2 checkpoint, which prevents movement into mitosis of cells with damaged chromatids, displayed normal activity. 98% of cells arrested mitosis after a dose of 1.5 GY of gamma rays. However, with aging there was a continuous emergence of cells that failed to delay mitosis. Within 30 population doublings, over 50% of cells were defective in their G_2 checkpoint. Cytogenetic analysis confirmed that cells

with attenuated G_2 checkpoint exhibited high frequencies of chromosome breakage in mitosis. E6-transformed cells displayed increased frequencies of aneuploidy and endoreduplication suggesting that the mitosis-dependency checkpiont also was attenuated. The results indicate that inactivation of p53 induces a condition of genetic instability and genotoxic stress that selects for outgrowth of cells with additional checkpoint dysfunction and even more severe genetic instability. This genetic destabilization occurs before immortalization and may contribute to derepression of telomerase. Supported by NIH grant CA42765.

1360 VULNERABILITY TO CHEMICAL CARCINOGENESIS IN EARLY S PHASE AND POSSIBLE GENETIC MECHANISMS FOR THIS SUSCEPTIBILITY

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Cells in synchronized cell proliferation in vitro or in vivo are most susceptible to malignant transformation when they are treated with chemical carcinogens during the early part of the S phase of the cell cycle. DNA is preferentially damaged when it is replicating, particularly when it is at the junctional region of replication forks. This suggested that DNA that is being replicated during the early S phase should include a significant target(s) for cell transformation. Methods were developed to assess the time sequence of replication of genes during S phase. Results showed that genes replicate in a specific temporal order in normal human fibroblasts. Based on these techniques a method was developed to isolate DNA enriched in sequences replicated at the start of S phase. DNA replicated in the earliest part of S phase was enzymatically restricted and cloned to form subgenomic DNA libraries. Clones from subgenomic libraries were analyzed to verify their early S phase replication, to evaluate their sequence characteristics, and to determine whether they function as origins of DNA replication. Concurrently, sites of earliest replication on human chromosome 16 were identified by screening a chromosome 16 library. Sites of earliest replication are being sub-cloned, sequenced and localized within the chromosome by fluorescent in situ hybridization. In parallel, chromosomal sites of earliest replication are being identified based on sites of incorporation of bromodeoxyuridine at the start of the S phase as seen in chromosomal bands in karyotypes. The sites of the earliest DNA replication might be important in carcinogenesis because they may include a regulatory region controlling entry into S phase. Alterations of the DNA sequence at such sites might reduce this regulation of entry into S phase, yielding the dysregulated progressive cell proliferation that typifies malignancies. Support by NIH grant CA42765.

1361 IMMUNOTOXICITY OF MEDICAL DEVICES: AN OVERVIEW

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The ability of a medical device to interact with the immune system currently involves assessment of the immunogenic potential and biocompatibility of the device or an extract of the device. However, implants are often in the body for extended periods of time and/or are placed by a surgical procedure that in and of itself will generate an acute inflammatory response. This symposium will discuss studies that have been performed to evaluate: the immunogenicity of various devices that consist of several different compositions (i.e., silicone, metals, and latex) in contact with different anatomical sites; the ability of a device to modulate an inflammatory response generated by a surgical procedure or trauma; and the response of the body to a material left in place for extended periods of time. This symposium will bring together scientists from many different disciplines to begin to identify and fill in the gaps in this area.

1362 BIOLOGY OF DEGRADATION PRODUCTS FROM ORTHOPAEDIC BIOMATERIALS

J. J. Jacobs, A. S. Shanbhag, J. Black, and T. T. Glant. *Rush Medical College, Chicago, IL.* Sponsor: J. Zelikoff

Cemented and cementless hip and knee arthroplasty is commonly employed in the treatment of arthritic disease. The costs of hip and knee prostheses were $14.5 billion in 1992. While it is estimated that the life span of these joint replacements is about 15– 20 years for elderly patients, it is often less for younger, more active patients. In 1992 approximately 5–10% of the surgical procedures for knee and hip prostheses were revisions due to failure of the original prostheses. The failures most commonly result from inflammation and bone loss induced by wear debris particles that accumulate in the tissues surrounding the prosthesis. The composition of particles causing these inflammatory changes and subsequent osteolysis include polymethylmethacrylate (bone cement particles), Ti-6A1–4V alloy, Co-Cr-Mo alloy, stainless steel and ultrahigh molecular weight polyethylene. The size of the particles that cause the problematic inflammatory reaction is approximately 0.5–1 um - generally within the range of other toxicologically significant particulates. These phagocytosable particles interact with macrophages, fibroblasts and osteoblasts within the periprosthetic tissues and have a variety of effects on these cells including stimulation of the release of "bone-resorbing cytokines" (interleukin 1 and interleukin 6), matrix metalloproteinases (collagenase and stromelysin), prostaglandin E_2 and suppression of the synthesis of type I and type II collagen precursors. These cells respond to particle challenge in a size, composition and dose dependant manner.

1363 IMMUNOTOXICOLOGY OF DEVICES USED IN ADHESION PREVENTION

K E Rodgers. *USC, Los Angeles, CA*

Adhesions are fibrous bands which unite two surfaces that are normally separate and are associated with chronic pain and distortio of organs. Currently, adhesion formation is reduced via the placement of a biodegradable barrier of knit oxidized regenerated cellulose (INTERCEED) between the organ surfaces expected to form adhesions. Hyskon [a 32% solution of Dextran 70], although not approved for intraperitoneal use, and a permanent barrier of expanded polytetrafluorethylene (Gore-Tex or Preclude), approved for peritoneal reconstruction, have also been used to reduce adhesion formation. Under clinical investigation for adhesion prevention is precoating with a liquid barrier of hyaluronic acid (HA). Each device is used intraperitoneally at the time of surgery and has unique considerations with regards to changes in the inflammatory response after surgery. INTERCEED is degraded within 5–7 days after placement and this process may involve macrophages. Hyskon causes hydroflotation and a small number of patients have allergic reactions. Preclude is a permanent barrier which has the potential for the generation of foreign body reaction. HA is used to precoat the area of surgical injury and may reduce the amount of trauma to the tissues. Current knowledge as to the immunotoxic effects of these various medical devices will be discussed.

1364 IMMUNOLOGICAL INVESTIGATIONS OF SILICONE IMPLANT MATERIALS

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Spearheaded by the breast implant controversy, the biocompatibility of silicone implant materials is being actively investigated at the clinical and nonclinical level. A primary focal point of these studies has been the potential interactions of silicones and the immune system and whether immune-mediated disease is linked to systemic silicone exposure. As part of our nonclinical research efforts, we have conducted a number of immunological studies with principal components of silicone implant devices; polydimethylsiloxane (PDMS) fluid, silicone gel and silicone elastomer. The areas of interest have included the NTP Tier I and II immunotoxicity batteries to determine whether silicone exposure can cause injury to the immune system, immunological sensitization models, and studies to characterize what exposure conditions are necessary for silicones to serve as immunological adjuvants. The results of these ongoing investigations will be compared to the research findings of other investigators and integrated with current clinical and epidemiological studies related to silicone implant devices.

1365 HYPERSENSITIVITY TO LATEX PROTEINS: ETIOLOGY, DIAGNOSIS AND PREVENTION

V J Tomazic. *Center for Devices and Radiological Health, Food and Drug Administration, Rockville, MD.* Sponsor: K. Rodgers

Increasing frequency and severity of allergy to latex proteins are in part the result of an almost ten-fold increase in use of medical gloves in the last

decade. The symptoms of allergic reactions range from local urticaria to anaphylaxis. Approaches to alleviate the problem involve identification of high-risk populations, identification of specific allergen(s), development of diagnostic methodology, and prevention. Multiple laboratory efforts to identify allergenic latex proteins are generating a diversity of results. The major protein bands recognized by immunoblotting were found in the molecular weight range from 2–100kD, but the results varied probably due to the sources of latex proteins and the test sera evaluated. Our studies of a number of latex extracts from various sources, including raw latex and latex products, revealed the major allergenic proteins in the range of 20–45kD. Evaluation of sera from latex-sensitive individuals confirmed that the specificity of antibodies differs from individual to individual, and may depend on the source of sensitization. It appeared that individuals sensitized with similar products may develop a similar response pattern. The individuals allergic to crossreacting foods may also have a specific pattern of the response. Identification of the major allergen(s) in latex is critical for the diagnosis of latex allergy and the assessment of allergen content in finished latex products. The correlation of the specific patterns of IgE response with the medical history and clinical symptoms may be a valuable parameter in diagnostic and preventive efforts.

1366 EFFECT OF PROSTHETIC WEAR DEBRIS ON MACROPHAGES, FIBROBLASTS AND BONE CELLS

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Biological response to wear debris generated by components of orthopaedic implants play a major role in prosthetic survival. In patients with implants undergoing aseptic loosening, the periprosthetic tissue is infiltrated with activated macrophages, fibroblasts and lymphocytes. Aseptic loosening is also characterized by osteolysis. The mechanisms by which wear debris play a role in the pathogenesis of aseptic loosening are unclear. The purpose of our study was to characterize cellular response to wear debris. Prototypes of tissue cells that are documented to mediate inflammatory and immunological response in the joint (macrophages, synovial fibroblasts, lymphocytes) and bone cells were used as "target" indicator cells. Debris (metal alloy, ultra high molecular weight polyethylene [UHMWPE]) was retrieved from tissues during prosthetic revision surgery and was solated by enzymatic procedure followed by ultracentrifugation. Polymethy-methacrylate cement (PMMA) and ceramic implant coating obtained from commercial sources were also tested. Target cells were exposed to particles at 37°C, 5% CO_2 for 1–2 days in monolayer or microcarrier spinner culture. The effect of wear debris on cells was assayed for (1) cytotoxicity, (2) ability to induce inflammatory cytokines (IL-1, TNFα, IL-6), (3) effect on protein synthesis. Cytotoxicity induced by CrCo, TiAlVa, UHMWPE, PMMA was dose dependent indicated by reduction of ^{3}H-thymidine proliferative indices. Exposure to large amounts of debris (10 mg/ml) resulted in few pyknotic adherent cells. Lower concentrations of debris (< 10mg/ml) which were not cytotoxic induced greater amounts of IL-1 and TNFα suggesting active secretion of inflammatory cytokines. Bioglass was less toxic than the other biomaterials tested but it induced cytokine release. We evaluated the effect of wear debris on tissue cells using systems that mimic some features of the biomechanical environment in the joint. These studies may yield insights into the in vivo behavior of tissue cells during the pathogenesis of aseptic loosening.

1367 IDENTIFICATION OF TWO Ah RECEPTOR (AhR) GENES IN THE TELEOST *FUNDULUS HETEROCLITUS*

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The mammalian AhR is a ligand-activated transcription factor that mediates dioxin effects. Comparative and phylogenetic studies of the AhR in non-mammalian species may contribute to an understanding of its normal physiological function. AhR proteins are expressed in livers of most vertebrate animals, including bony and cartilaginous fish (*Arch. Biochem. Biophys.* **310**: 218 (1994)). We are characterizing the AhR in the killifish *Fundulus heteroclitus*. Reverse-transcription-polymerase chain reaction (RT-PCR) amplification of *Fundulus* hepatic RNA using degenerate primers produced a 690-bp fragment containing the PAS domain of a putative *Fundulus* AhR (*Biochem. J.* **310:** 383 (1995)). The deduced amino acid sequence of this fragment shared 62–64% identity with mammalian AhR sequences, and lesser identity with the other PAS proteins Sim, Per, ARNT, and HIF-1α. Screening of a *Fundulus* genomic DNA library with a mouse AhR cDNA and with the *Fundulus* RT-PCR product identified several positive clones. Sequencing of these clones revealed two distinct AhR-like genes. The sequence of one of these was identical to that of the RT-PCR product, while the other was more closely related to mammalian AhRs (68–69% amino acid identity over ~200 residues). Both AhR forms were expressed in liver, as determined by RT-PCR using specific primers, followed by sequencing of the amplified products. Further analysis of these two possible AhR genes may reveal and illuminate novel functions of vertebrate Ah receptors.

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1368 TWO-DIMENSIONAL GEL ELECTROPHORETIC ANALYSIS OF THE Ah RECEPTOR NUCLEAR TRANSLOCATOR

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The *Ah* receptor (AhR) and the *Ah* receptor nuclear translocator (Arnt) are capable of forming a transcriptionally active dimeric complex. The biochemical events that are required for dimerization and transactivation are not fully understood. The purpose of this study was to determine whether phosphorylation or other covalent modifications occur between Arnt existing in the monomeric form and after heterodimerization with the AhR and subsequent binding to DNA. Mouse hepatoma cell line 1c1c7 cytosol was subjected to two-dimensional gel electrophoresis, followed by blotting to membrane. Arnt was visualized with monoclonal antibody 2B10, results revealed that Arnt has a considerable level of charge heterogeneity. The pI range is 5.7–6.4, with the predominate form at a pI of 6.2. The AhR/Arnt dimer was immunoprecipitated from high salt nuclear extract obtained from Hepa 1 cells treated with β-naphthoflavone using an anti-AhR polyclonal antibody. This immunoprecipitate was subjected to two-dimensional gel electrophoresis and co-precipitated Arnt was visualized on a protein blot. The results indicated that Arnt complexed with the AhR in the nucleus has an isoform pattern shifted toward the basic end. Thus a significant shift in pI occurs during the dimerization and/or after binding to DNA.

1369 CHARACTERIZATION OF THE DNA BINDING FORM(S) OF THE Ah RECEPTOR COMPLEX

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The Ah receptor (AhR), a ligand-dependent transcription factor, binds with high affinity to a specific DNA recognition site (the dioxin responsive element (DRE)). The binding of the transformed AhR complex with the DRE is the first step in the activation of gene expression. To examine transcriptional activation by the AhR, the protein subunit composition of the complex must be established. It is believed that transformed AhR complexes exist as a heterodimer of the AhR and ARNT (Ah receptor nuclear translocator). Recent evidence suggests the involvement of other AhR subunits/factors. In previous crosslinking studies, we demonstrated that the transformed 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD):AhR complex exists as two distinct DNA binding forms, containing at least three protein subunits (97, 105 and 115 kDa) which inducibly bound to a DRE oligonucleotide. Here we have characterized these three protein-DNA cross-linked complexes using antibody recognition and precipitation experiments. Guinea pig hepatic cytosolic TCDD: AhR complexes, transformed *in vitro* were UV-crosslinked to a radiolabeled BrdU-containing DRE oligomer. Immunoprecipitation with an ARNT antibody identified the 97 kDa protein as ARNT. Western blots using an AhR antibody confirmed the identity of the 105 kDa protein as the AhR. The lack of recognition of the 115 kDa protein-DNA complex by either antibody suggests that it represents a unique protein which is associated with the DNA binding form of the AhR. The identity and functional activity of this protein remains to be elucidated. Supported by ACS (CN-4C) and NIEHS (ESO7072).

1370 ACTIVATION OF OXIDATIVE STRESS-MEDIATED TRANSCRIPTION FACTORS BY TCDD

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), the prototype aromatic hydrocarbon receptor (AHR) agonist, is a potent tumor promotor and complete liver carcinogen. Since TCDD is nonmutagenic, it may promote neoplasia via altered oncogene or tumor suppressor gene expression. The underlying mechanisms and signal transduction pathways involved in this TCDD effect remain uncertain. *In vivo*, especially female rats, TCDD produces indicators

of oxidative stress, including lipid peroxidation and oxidative DNA damage. In cultured cells, we find that TCDD does not affect biochemical indicators of oxidative stress (lipid peroxidation, soluble thiol levels, and protein thiols or carbonyls). However, two transcription factors known to be stimulated via the oxidative stress signal transduction pathway, NF-kB and AP-1, are activated by TCDD in wild type mouse liver tumor cells (Hepa-1), but not in the two Hepa-1-derived mutant cell lines, AHR-deficient cells (*c2*) and CYP1A1 enzyme-defective cells (*c37*). In Hepa-1 cells, TCDD produced an overexpression of AP-1 for more than 72 h; furthermore, this TCDD-mediated activation of NF-kB and AP-1 was diminished by the thiol antioxidant *N*-acetylcysteine. These results suggest that TCDD stimulates oxidative stress signal transduction and the resulting transcription factor activation, by a pathway involving the AHR and a functional CYP1A1. (Supported by NIH P30-ES06096, RO1-ES06273 and RO1-AG09235)

1371 EVIDENCE FOR A DISTINCT DNA-BINDING FORM OF THE ARYL HYDROCARBON RECEPTOR (AhR) IN HUMAN B LYMPHOCYTES

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2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) suppresses proliferation and immunoglobulin production in murine and human B lymphocytes. We previously reported that the AhR complex was constitutively present in nuclear extracts from two human B lymphocyte cell lines (BBRC 212: 27, 1995). The present study compared the AhR complex in the IM-9 and PJS-91 human B lymphocyte and HepG2 human hepatoma cell lines. Cytochrome P-450 1A1 was induced by TCDD only in the PJS-91 cell line, but at considerably lower levels than in HepG2 cells. IM-9, PJS-91, and HepG2 cells expressed identical AhR and ARNT mRNA transcripts. The three cell lines also expressed similar immunoreactive AhR and ARNT protein in cytosol and nuclear extracts. Although AhR mRNA and protein levels were much lower in IM-9 and PJS-91 cells, ARNT mRNA and protein levels were similar between IM-9, PJS-91, and HepG2 cells. In gel shift assays, the cytosolic AhR in IM-9 and PJS-91 cells was indistinguishable from the HepG2 cytosolic and nuclear DNA-binding AhR complex. The AhR complex in IM-9 and PJS-91 nuclear extracts consisted of several closely migrating bands; only one being recognized by an AhR antibody, while an ARNT antibody reacted with all bands. Protein:DNA cross-linking analysis revealed the presence of a novel DRE-binding protein in nuclear extracts from IM-9 and PJS-91 but not HepG2 cells. These results show that IM-9 and PJS-91 human B cells constitutively express a distinct nuclear DNA-binding form of the AhR complex that may result from the presence of an additional protein or a structural variant of the AhR.

1372 EXPRESSION OF K-RAS PROTEIN IN DIOXIN-TREATED MOUSE LUNG EPITHELIAL CELLS AND LUNG TISSUES

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The halogenated aromatic hydrocarbon, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) poses serious concern as a persistent environmental contaminant. TCDD is a potent promoter of rodent liver, lung, and skin tumors. Its possible contribution to human cancer risk has received much attention and can be better understood if its mechanism of action is elucidated. Since alterations in signal transduction pathways are implicated in the process of carcinogenesis an attempt has been made to see how TCDD alters expression of key components of signalling events, in particular the protoncogene c-*K-ras*, which is often found to be mutated in mouse lung tumors. The expression of K-ras p21 protein was studied in TCDD treated mice lung tissues and E10 mouse lung epithelial cells by immunoblotting and immunohisto-chemical staining with a K-ras specific antibody. Lungs of Swiss male mice, treated with a single dose of 5 nM TCDD, showed a ~4-fold increase in membrane-bound K-ras 48 hrs after treatment. In E10 cells treated with 10 nm TCDD, a ~2-fold increase in the amount of p21 ras protein was seen particularly in the membrane fraction, 72 hrs after dosing with TCDD. Immunostaining of E10 cells characteristically highlighted the perinuclear vacuoles and points of cell contact in addition to a generalized increase in cytoplasmic staining. Longer time points are in progress. The data thus far are consistent with TCDD promoting lung tumorigenesis by causing overexpression of K-ras protein.

1373 ROLE OF FAS APOPTOSIS AND MHC GENES IN 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)-INDUCED IMMUNOTOXICITY OF T CELLS

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TCDD is well known for its immunotoxic effects, although the exact mechanism is not known. In the current study, we investigated the role of Fas (CD95), an important molecule involved in apoptosis, on TCDD-mediated immunotoxicity using mice bearing homozygous lpr mutation which leads to failure of expression of Fas. When TCDD was administered orally at 0, 0.1, 1.0 or 5.0μg/kg body weight for 11 days, it was found to be less toxic to the thymocytes from C57BL/6 lpr/lpr mice (Ah-responsive, Fas) when compared to C57BL/6+/+ mice (Ah-responsive, Fas^+). Similar results were obtained when peripheral T cell responsiveness to antigenic challenge with conalbumin was studied. Using mice that differed only at the MHC, it was noted that B10.D2 (Ah-responsive, $H\text{-}2^d$) were more sensitive to TCDD-mediated thymic atrophy and peripheral T cell dysfunction when compared to B10 mice (Ah-responsive, $H\text{-}2^b$). In all TCDD-sensitive strains tested, the thymic atrophy was accompanied by a uniform depletion of all four subset of T cells ($CD4^+$, $CD4^+CD8^+$, $CD4^-CD8^-$ and $CD8^{3)+}$). Furthermore, in these strains, TCDD suppressed the antigen-specific peripheral T cell responsiveness but not the responsiveness of naive resting T cells to polyclonal mitogens. Lastly, using cell-mixing experiments, it was demonstrated that TCDD directly affected the T cells responding to conalbumin but not the antigen presenting cells. Together, our studies demonstrate that although Ah locus plays the primary role determining the toxicity of TCDD on the T cells, there are secondary factors such as expression of Fas or the MHC phenotype which may play an important role in TCDD-mediated immunotoxicity. The role of Fas further suggests that TCDD may induce toxicity in T cells by triggering apoptosis.

1374 IDENTIFICATION OF A MOTIF WITHIN THE 5′ REGULATORY REGION OF pS2 WHICH IS RESPONSIBLE FOR AP-1 BINDING AND TCDD-MEDIATED SUPPRESSION

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TCDD is able to modulate the endocrine system through a number of mechanisms. Previous studies of the human breast cancer prognostic marker, pS2, have demonstrated that TCDD is able to suppress 17ß-estradiol (E2)-induced expression of pS2-regulated luciferase reporter genes. This activity was localized to a DRE-like motif (iDRE) located within the 5′ regulatory region of pS2. Further examination of this site have revealed the presence of a putative AP-1 site located immediately 3′ to the motif required for TCDD-mediated suppression. This putative AP-1 site diverges by one base pair from the consensus AP-1 site. Gel shift assays performed using nuclear extracts prepared from TPA-treated MCF-7 cells have demonstrated an induced complex binding to the AP-1 site. This complex can be competed by the oligonucleotides containing either the iDRE or consensus AP-1 site but not by oligonucleotides containing iDRE or AP-1 sites with known mutations that do not bind transcription factor. Antibodies were used to supershift the TPA-induced complex, confirming the presence of *c*-Jun within the complex. Transient transfection assays using luciferase reporter gene constructs which retained the iDRE but critically mutate the putative AP-1 binding site were used to demonstrate the importance of this AP-1 binding motif. In MCF-7 cells, constructs that contained a mutated AP-1 motif showed greatly diminished E2-inducibility relative to the wild type constructs. Co-treatment of AP-1 knockouts with E2 and TCDD had no effect on the inducibility compared to E2 alone. These results suggest that an interaction between AP-1 and factors bound to the iDRE may contribute to TCDD-mediated inhibition of E2-induced pS2 gene expression.

1375 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) AS AN ANTIESTROGEN IN MCF-7 HUMAN BREAST CANCER CELLS: INHIBITION OF ESTROGEN-INDUCED HEAT SHOCK PROTEIN 27 GENE EXPRESSION

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MCF-7 human breast cancer cells are estrogen-responsive and express relatively high levels of the estrogen receptor (ER). 2,3,7,8-Tetrachlorodibenzo-

p-dioxin (TCDD) inhibits E2-induced cell proliferation, progesterone receptor binding, secretion of tissue plasminogen activator and the 160-, 52-, and 34-kDa proteins. Treatment of MCF-7 human breast cancer cells with 10 nM E2 resulted in a 2-fold induction of heat shock protein 27 (Hsp27) mRNA levels whereas 10 nM TCDD alone did not affect expression of this gene. In cells cotreated with both E2 plus TCDD, the E2-induced Hsp27 mRNA levels were inhibited within 2 hr after addition of TCDD and this inhibitory response persisted for up to 24 hr. The 5'-proximal region of the gene was further investigated to identify genomic sequences associated with both E2-responsiveness and the inhibitory effects of TCDD. An Sp1-ER binding site, $GGGCGGG(n)_{10}GGTCA$, was identified at -105 to -84 and formation of the Sp1/ER complex was confirmed in *in vitro* assays using synthetic Hsp27-[^{32}P]Sp1/ERE oligonucleotides in gel electrophoretic mobility shift assays and in transient transfection assays with a plasmid containing an Sp1/ERE sequence cloned upstream to a thymidine kinase promoter and a reporter gene. Current studies are investigating the location of *cis*-genomic sites responsible for the TCDD-induced inhibitory response.

1376 TRANSCRIPTIONAL DOWNREGULATION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-4 BY 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN IN MCF-7 AND T47D HUMAN BREAST CANCER CELL LINES

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Insulin-like growth factor (IGF) is an important mitogen in human breast cancer cells and is modulated by the interaction with IGF binding proteins (IGFBPs). The IGFBP family consists of 6 proteins which bind to IGF and either enhance or decrease binding of IGF to its cognate receptor. Previous studies have shown that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) inhibits E2-induced responses in estrogen receptor-positive (ER^+) breast cancer cells, and this effect is mediated through the aryl hydrocarbon receptor (AhR). The effect of TCDD on IGFBP-4 secretion was investigated in MCF-7 and T47D cells by Western ligand blot analysis using [^{125}I]IGF. IGFBP-4 secretion was induced approximately 2-fold by 1 nM E2 in both MCF-7 and T47D cell lines; 10 nM TCDD downregulated basal levels of the secreted protein, and in cotreated cells, the E2-induced secretion was also inhibited. In MCF-7 cells, TCDD also decreased basal and E2-induced IGFBP-4 mRNA levels, and the inhibitory responses were observed within 2 hours of treatment with TCDD. The role of the AhR in mediating these responses was also investigated in Ah-nonresponsive benzo[a]pyrene-resistant MCF-7 and T47D variant cells. Surprisingly, the variant cells secreted 80-and 13-fold lower levels of the IGFBPs, and these cell lines are currently being used as models to understand the function of IGFBPs in human mammary cancer cells.

1377 CIGARETTE TAR DOES NOT PROMOTE ARTERIOSCLEROTIC PLAQUE DEVELOPMENT

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We reported previously that a) inhalation of either mainstream or sidestream cigarette smoke (CS) promotes aortic arteriosclerotic plaque development; b) butadiene, a vapor-phase component of sidestream smoke, promotes plaque development at 20 ppm, only 2X higher than the threshold limit value; and c) individual tar fraction carcinogens in CS either do not promote plaque development or do so only at high concentrations. Here we asked whether exposures to concentrated CS tar promote plaque development. Mainstream CS tar, extracted (acetone/dry ice) from burning, unfiltered reference cigarettes, was solubilized in dimethylsulfoxide (DMSO) and injected (25 mg/kg;i.m.) into cockerels, 1X/wk. Negative controls were injected weekly with DMSO and positive controls with 7, 12 dimethylbenz(a)anthracene (DMBA; 10 mg/kg/wk). Cockerels were sacrificed at 20–21 weeks of age, after 16 weekly injections. Plaque location and prevalence did not differ from group to group. Morphometric analysis of plaque cross-sectional areas showed that plaque sizes, which are log-normally distributed, were significantly larger in the DMBA cockerels compared to both the TAR and DMSO groups. There were no significant differences in plaque size between DMSO and TAR cockerels. Although CS is clearly the single greatest known environmental cause of cancer, as well as a major contributor to heart disease, there is little evidence that the tar fraction is the primary source of environmentally relevant, CS toxicants. The results reported here, combined with our previous findings, suggest that the major health threatening components of CS are in the vapor phase.

1379 COVALENT BINDING AND ONE-CARBON INCORPORATION OF ^{14}C- CL 283,796 (ACYL-COA-ACETYL TRANSFERASE-INHIBITOR) IN VIVO IN HUMANS AND DOGS

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Sponsor: G. Fisher

^{14}C-CL 283,796 (Al) administration in dogs (D) and humans (H) results in persistent radioactivity in plasma with half-lives between 12 and 22 days. One possible reason for the prolonged residence is covalent binding of a metabolite(s) to proteins. Another possibility is the incorporation of ^{14}C into the endogenous one-carbon pool *via* the urea cycle. Mass-balance studies in humans showed that 1% of the administered radioactivity was expired as CO_2, indicating a very limited release of ^{14}C-carbon during metabolism.

To characterize the plasma radioactivity, D or H plasma was exhaustively extracted with organic solvents before and after digestion of proteins. Little or no radioactivity was extracted. Furthermore, heating (80°C), denaturing (sodium dodecyl sulfate or guanidine, to unfold proteins), in combination with the alkylating agent iodoacetamide (to prevent refolding) also did not release any radioactivity. These results suggest that the binding is not due to non-covalent interactions of parent compound, but due to covalent/irreversible binding of a metabolite(s) or due to one-carbon incorporation of ^{14}C-carbon into proteins.

To determine whether the plasma radioactivity was due to one-carbon incorporation, we analyzed the urea in urine obtained from D and H for radioactivity derived from Al. ^{14}C-Sodium bicarbonate(SB) dosed D were used as a positive control. Some radioactivity was found in the urea indicating some one-carbon incorporation of Al. Nevertheless, the specific activity of the urea carbon in SB dosed D was 37.4 and 22.3 fold higher than Al dosed D and H respectively. When ratios of urinary urea specific activity versus plasma radioactivities were compared, the ratio for SB dosed D was approximately 90 and 150-fold higher than Al dosed D and H respectively. In addition, amino acid analysis of purified radioactive fractions, following digestion of proteins, did not contain significant levels of amino acids. These results indicate that one-carbon incorporation accounts for only a minor portion of the circulating radioactivity, with the majority of the radioactivity presumably bound covalently to plasma proteins.

1380 EFFICACY OF A SHORT-ACTING METHEMOGLOBIN FORMER IN PREVENTING CYANIDE INTOXICATION

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Current therapy for cyanide intoxication is iv administration of sodium nitrite and sodium thiosulfate. Prophylaxis treatment for at-risk populations is desired to overcome constraints of current therapy. One approach is to administer a compound that induces a level of methemoglobin (MHb) that prevents CN^- from binding to the cellular respiratory enzyme, cytochrome oxidase. This study was designed to determine the efficacy of para-aminopropiophenone (PAPP) in delaying cyanide intoxication in an anesthetized animal model using a nonlethal endpoint. PAPP doses of 0, 0.12, 0.17, and 0.37 mg/kg were selected to produce MHb levels that peaked at near 0, 2.5, 5, and 10 percent, respectively. The experiment was conducted in four periods using a cross-over design. During each period, each of the four doses was administered to two animals. NaCN infusion was initiated once the MHb level was predicted to be at the targeted levels (28, 28, 37, and 56 min). Infusion ceased 10 seconds after cessation of functional breathing (respiratory arrest). Blood samples were collected periodically to measure hemoglobin, MHb, and total blood CN^-. The primary responses measured were time to respiratory arrest, percent MHb, and amount of infused NaCN. Other data collected included heart and respiratory rates. The PO-NE-MAH® Digital Data Acquisition, Analysis, and Archiving System was used to collect data and measure these parameters. Average time to respiratory arrest and amount of infused NaCN of each PAPP-dosed group were significantly greater than those of the control group. Statistically significant MHb-response relationships were observed for time to respiratory arrest and amount of infused NaCN. [Supported by USAMRDC DAMD17-89-C-9050]

1381 ANTIMONY ALTERS KINETICS OF CALCIUM TRANSIENTS OF NEONATAL RAT CARDIAC MYOCYTES

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Cardiotoxicity has long been associated with exposure to antimony compounds. Effects in humans exposed to trivalent forms of antimony include hypotension, bradycardia, ventricular tachycardia, electrocardiographic changes and sudden death. The present study describes the effects of overnight exposure to a nonlethal concentration of potassium antimonyl tartrate (PAT) on calcium transients in neonatal rat cardiac myocytes. Isolated cardiac myocytes were cultured on glass coverslips and after 2 days were exposed overnight to 2 μM PAT in medium with 10% serum. Both control and PAT-treated cells were beating and indistinguishable in microscopic morphology. Myocytes loaded with the fluorescent probe fura-2 were paced at 0.5 Hz using field stimulation and $[Ca^{2+}]_i$ monitored on an inverted phase contrast microscope. The calcium transient was expressed as changes in the ratio of fluorescence at 340nm to 380 nm. For each cell, the average $[Ca^{2+}]_i$ transient was obtained from 15 individual transients. This was done for 3–5 cells per treatment in replicate experiments. Transients were characterized by 1) the basal "diastolic" ratio (D); 2) the peak amplitude of the ratio (A); and 3) the decrease in the ratio that follows A, as expressed by the rate constant of decay (k) that was determined by fitting the data to an exponential. Cells treated with 2 μM PAT exhibited a small increase in diastolic ratio (105% of control), a reduction in peak amplitude (73% of control), and a decrease in k (70% of control). Treatment of PAT-exposed and control cells with isoproterenol (10^{-7} M) altered the $[Ca^{2+}]_i$ transient of both groups consistent with a β-adrenergic effect. The effect of PAT on diastolic ratio was maintained (110% of control), whereas A and k were comparable between control and PAT-treated cells. The demonstrated effects of antimony on $[Ca^{2+}]_i$ transients in contracting myocytes could be a factor in the cardiac arrhythmias observed in humans exposed to antimony.

1382 PRINCIPAL COMPONENT ANALYSIS OF ENVIRONMENTALLY-ASSOCIATED ARTERIOSCLEROTIC PLAQUE DEVELOPMENT

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Heart disease is the single greatest killer in the U.S. The cockerel (young rooster) is a reliable animal model for investigating the promotional effects of environmentally-relevant levels of toxicants on arteriosclerotic plaque development, and the mechanism of action of these agents. Our analyses to date have concentrated on the effects of test agents on plaque development in the abdominal aortas from varying sized groups of treated vs. control animals. Comparisons were made on pooled data from each group. We have extended the analysis to compare responses of individual cockerels within and between groups. Data were analyzed from earlier studies with "second-hand" cigarette smoke, putative carcinogens in the smoke, other carcinogens and carbon monoxide as the test agents. Routes of exposure were by inhalation or injection. Principal component analysis revealed that the most important component of the data was the weighted average of plaque size. The most pronounced promotional effect of the active environmental agents was on plaques located in the distal two fifths of the abdominal aorta. The analysis distinguishes clearly between "responders" and "non-responders". The results also show that test groups with as few as ten cockerels each can be a reasonable size to determine whether toxicants accelerate arteriosclerosis at doses which do not cause other overt health effects.

1383 NON-LETHAL ENDOTOXIN (LPS) TREATMENT DECREASES CONTRACTION RATE AND INDUCES NITRIC OXIDE SYNTHASE ACTIVITY IN CULTURED RAT CARDIAC MYOCYTES

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Sepsis and endotoxic shock often result in myocardial depression. Recently, nitric oxide (NO) production by inflammatory cells and other tissues has been proposed to contribute to this phenomenon. In studies to determine the effect of cytokine stimulated NO production on cardiac myocytes, the inflammatory, cytokines TNF, IFN and IL-1 were found to increase nitrite in cell media over 48 h treatment. TNF, IL1, and IL6 increased contraction rates as did the NO synthase inhibitor NMA. Other researchers have shown that LPS-stimulated macrophages co-cultured with myocytes also increased nitrite production and decreased contraction rates. We investigated the effects of non-lethal concentrations of *Escherichia coli* endotoxin (LPS) without added cytokines on cultured cardiac myocytes. Parameters measured included- cytotoxicity, myocyte contraction rate, and NO synthesis as measured by nitrate and nitrite present in the culture media. The concentrations of LPS used were not overtly toxic to cells up to 24 h exposure (as measured by LDH release and cell attachment). Nitrite/nitrate in the media remained low at all concentrations from 0 – 4h and then rapidly increased in a concentration-dependent manner out to 24 h. Myocyte contraction rate followed a similar response pattern in that from 0 – 4h cells contracted at similar rates regardless of LPS concentration. After 6 h, contraction rates were observed to decrease in a time and concentration-dependent manner with contraction rate inversely correlated to media nitrate/nitrite concentrations. In summary, this research demonstrates that LPS-exposed cardiac myocytes generate NO independent of additional cytokine or other inflammatory/immunological stimulus.

1384 APPLICATION OF RT-PCR TO REGIONAL MAPPING OF β_1- AND β_2-ADRENERGIC RECEPTOR mRNAs IN RAT HEART

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Chronotropic and inotropic activities of the heart are stimulated through β_1- and β_2- adrenergic receptors (AR). Regional distribution of β-ARs and corresponding mRNAs in the heart are not well defined despite the potential for molecular phenotypic redistribution to be a factor in pathological conditions. Investigating regional distribution of specific mRNAs in the rat have been hampered by the paucity of tissue and the low abundance in heart of β-ARs. Reverse transcription in combination with the polymerase chain reaction (RT-PCR) was used to measure the number of β_1- and β_2-AR mRNA molecules in the four chambers of rat heart. The right atria was microdissected into four sections with the SA node limited to subsection RA/1. RT-PCR resulted in single amplification products of expected sizes. cRNAs specific for β_1- and β_2-ARs but with restriction sites produced by punctual mutations were used as internal standards. The mean number of β_1-AR mRNA molecules per ng of total RNA ranged from 663 ± 75 to 918 ± 90 in the ventricular and atrial regions. There was significantly more β_1-AR mRNA in right atria than left atria or ventricles. Right atrial subsections RA/1 and RA/2 contained significantly more β_1-AR mRNA than subsections RA/3 and RA/4. The number of β_2-AR mRNA molecules ranged from 375 ± 67 to 481 ± 104 in the regions examined. The number of β_2-AR mRNA molecules did not differ significantly among the chambers or subsections. The sums of β_1- and β_2-AR mRNAs among the individual chambers and atrial subsections were equivalent, but the β_1-/β_2-AR mRNA ratios ranged from 1.45 ± 0.08 to 2.49 ± 0.22 and varied significantly. The highest ratio occurred in the right atria and the lowest in left ventricle. Within the right atria, subsection RA/1 exhibited the highest ratio which was significantly greater than subsection RA/3, but not subsections RA/2 or RA/4. Comparison of the above variables between adult (3 mo) and senescent (24 mo) rats revealed significant phenotypic remodeling of β-AR mRNA distribution throughout the myocardium. These changes could contribute to increased rhythm disturbances observed in senescent heart.

1385 THE RELATIONSHIP BETWEEN PLASMA CONCENTRATIONS, HEMODYNAMIC EFFECTS, AND CARDIOVASCULAR (CV) TOXICITY IN DOGS TREATED WITH MINOXIDIL (MNX)

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The threshold hemodynamic changes associated with MNX-induced CV toxicity characterized by subendocardial necrosis, right atrial hemorrhage, and coronary vascular medical hemorrhage and necrosis in the dog have not been defined. To determine the relationship between plasma concentration, hemodynamic effects [heart rate (HR) and mean arterial pressure (MAP)] and CV toxicity, groups of female Beagle dogs were treated with a continuous IV infusion of dextrose (control), 0.05, 0.14, 0.43, 1.44 or 4.32 mg/kg/day of MNX for 3 days. Plasma concentration of MNX increased in a time-dependent dose related manner. The no significant effects doses (steady-state plasma concentrations of MNX) for hemodynamic effects and CV toxicity were 0.05 mg/kg (2.8 ng/mL) and 0.14 mg/kg (7.6 ng/mL), respectively. CV toxicity occurred at a steady-state plasma concentration of 16.6 ng/mL (0.43 mg/kg) where HR was increased by 51 beats/min and MAP was decreased

by 36 mm Hg. The absence of CV toxicity at 7.6 ng/mL although HR was increased by 44 beats/min and MAP was decreased by 16 mm Hg indicated that hemodynamic changes must be profound to induce CV toxicity in the dog. This study also suggested that hypotension may be more critical than tachycardia for the induction of MNX-induced CV toxicity in the dog. Because of the super sensitivity of the dog to the CV effects of MNX, the safety margin of MNX should be based on the invoked hemodynamic changes rather than on plasma concentration of MNX in dogs and humans.

1386 CARDIAC SENSITIZATION MODEL DEVELOPMENT USING BIOCHEMICAL MARKERS

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Chlorofluorocarbons (CFCs) are being phased out by international regulations because of negative effects on the ozone layer. Alternate fluorochemicals have been developed to replace the CFCs. Inhalation toxicology profiles have been conducted on those alternatives that are being developed for commerce. Both the CFCs and their replacement hydrofluorocarbons sensitize the dog heart to an intravenous epinephrine challenge following a ten minute exposure to the fluorochemical. This sensitization response occurs when multiple ectopic beats or atrial fibrillation is evident on electro cardiograms. The relationship between exposure levels and electrophysiological/ mechanical parameters was explored by detecting a biomedical marker in dog hearts using magnetic resonance spectroscopy imaging and magnetic resonance spectroscopy techniques. The purpose of this research was to initiate the background data for developing a new model for cardiac sensitization.

1387 EXPOSURE TO OZONE ALTERS THE ADHESIVE PROPERTIES OF ALVEOLAR MACROPHAGES

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The inflammatory response in the lung following acute exposure to ozone (O_3) is mediated by several cell types and molecules associated with or released by those cells. In this study, rats were exposed to 0.8 ppm O_3 for 3 hrs and macrophages were recovered by bronchoalveolar lavage. These cells were analyzed for adherence to adult rat lung (ARL-14) epithelial cells and surface expression of the integrin, CD11b, and intracellular adhesion molecule-1 (ICAM-1). The adherence of macrophages from O_3-exposed rats to ARL-14 cells (35 $\pm$ 3%) was found to be significantly greater than that of cells recovered from air-exposed controls (17 $\pm$ 2%). Furthermore, treatment with neutralizing antibodies to CD11b, ICAM-1, or interleukin-1α (IL-1α) and tumor necrosis factor-α (TNF-α) in combination significantly reduced this adhesive response. A modest but significant increase in surface expression (peak channel) of CD11b, but not in ICAM-1, was detected in macrophages from O_3-exposed rats (15.8 $\pm$ 0.5) as compared to air-exposed rats (12.4 $\pm$ 0.4). These data suggest that IL-1α, TNF-α, CD11b, and ICAM-1 all play functional roles in the O_3-induced adherence of lavageable macrophages, but that these functional changes involve only slight differences in the surface expression of cell adhesion molecules. Supported by NIEHS grant ESO3521. ACP is supported by a fellowship from USEPA and NIEHS training grant 5T32ESO7157.

1388 GLUTATHIONE (GSH) RESYNTHESIS IN TRACHEOBRONCHIAL AIRWAYS IN RATS CHRONICALLY EXPOSED TO OZONE

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Gaseous pollutants such as ozone cause a highly focal lesion in the lung involving the distal trachea and centriacinar regions. Following chronic exposure, these areas become resistant to further injury. Earlier work demonstrating a lack of consistent correlation between regions of high O_3 susceptibility in the lung with low activities of antioxidant enzymes or with low levels of GSH suggested that the rates of GSH resynthesis might be a critical factor in determining target site selectivity. Accordingly, we have examined the rates of GSH resynthesis in trachea, distal bronchiole and parenchyma of filtered air and O_3 exposed (90 days, 1 ppm) rats. GSH was depleted from airway subcompartments with diethyl maleate and the rates of GSH recovery were examined in depleted airways incubated in medium containing cysteine, methionine, GSH or cystine. GSH levels returned to control values within 1 hr when airway subcompartments were incubated with medium containing cysteine. In trachea and distal bronchioles, GSH levels returned to those observed initially while in the parenchyma, GSH levels rebounded to twice the original levels. The rates of GSH resynthesis did not vary with airway subcompartment nor were the rates different in filtered air compared to O_3 exposed animals. Addition of cystine, GSH or methionine to the medium increased intracellular GSH levels but the rate of repletion was not as rapid as with cysteine. These studies suggest that the focal lesion caused by ozone and the tolerance that ensues after chronic O_3 exposure is not related to altered rates of GSH synthesis in target lung areas. Supported by NIEHS 00628 and ES 05707.

1389 SUPEROXIDE DISMUTASE (SOD) AND CATALASE INCREASE INDUCIBLE NITRIC OXIDE (iNO) PRODUCTION BY RAT ALVEOLAR MACROPHAGES (AM)

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iNO has been implicated in cytotoxic reactions which occur in the presence of reactive oxygen species. However, NO also functions as an antioxidant in vivo. In the present study, we investigated the effect of SOD and catalase, antioxidant enzymes, on the regulation of iNO production by rat AM. AM were obtained by bronchoalveolar lavage from normal male rats and cultured for 1 hr before exposure to test agents. Media nitrate and nitrite (NOx) were used to index NO production and Northern blot analysis was used to detect iNO synthase (iNOS) mRNA. Incubation of AM with SOD (8,000 U/ml) or catalase (20,000 U/ml) for 18 h resulted in increases in NOx, as well as detectable iNOS mRNA levels. Exposure of AM to SOD or catalase for only 1 or 3 hr was sufficient to increase in NOx 18 hr later. Addition of pyrollidine dithiocarbamate (PDTC; 5uM) or cyclohexamide (CX; 10 uM) at the same time as SOD and catalase abolished increases in NOx. However, PDTC or CX were ineffective when added 6 hr after antioxidant exposure. These results suggest that SOD and catalase can directly stimulate the production of iNOS via a transcriptional mechanism. Antioxidant enzymes may be protective by selectively decreasing oxidant tone and increasing NO production.

1390 EFFECT OF WELDING FUME ON RAT ALVEOLAR MACROPHAGE VIABILITY, RESPIRATORY BURST, AND TUMOR NECROSIS FACTOR-α RELEASE

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We compared the *in vitro* responses of alveolar macrophages (AMs) to welding fumes assessing viability, respiratory burst, and tumor necrosis factor-α (TNF-α) release. Fume was collected during fluxcored manual metal arc (MMA) and gas metal arc (GMA) welding using two consumable electrodes: stainless steel (SS) or mild steel (MS). The elemental constituents of the fume was identified by energy dispersive spectroscopy: 1. MMA-SS: 22.3% K, 19.4% Fe, 13.1% Cr, 12.6% Si, 8.2% Ca, 8.0% Mn, 5.0% Na, 4.3% Ti, 8.1% other; 2. GMA-SS: 52.3% Fe, 22.2% Cr, 18.3% Mn, 4.9% Ni, 2.3% Si; 3. GMA-MS: 89.2% Fe, 8.2% Mn, 2.6% Si. AMs were recovered from CD/VAF rats by bronchoalveolar lavage, incubated with the fume (0.025 and 0.10 mg/ml) suspended in saline, and then assayed 30 min, 12 and 24 hr later. Following a 30 min incubation at 0.025 mg/ml, the respiratory burst increased 26.7%, 17.2%, and 7.9% above control (AMs exposed to no particles) in AMs exposed to MMA-SS, GMA-SS, and GMA-MS fumes, respectively. After 12 hr at the same concentration, a significantly elevated ($p<0.05$) amount of TNF-α was released from the AMs when incubated with the MMA-SS fume as compared to the other fumes. Following a 24 hr incubation with a concentration of 0.10 mg/ml, the MMA-SS fume caused a 61.2% loss of AM viability as compared to a 42.5% and 39.7% loss from the GMA-SS and GMA-MS fumes, respectively. We have demonstrated that SS fumes were more toxic than MS fumes, consistent with higher non-ferrous constituents in SS. The SS fume from MMA welding had the greater effect on AM function, probably due to fluxes used in MMA. (American Welding Society, NIOSH#109979)

1391 ROLE OF IRON IN THE GENERATION OF RADICALS BY FRESHLY FRACTURED SILICA: AUGMENTATION OF PULMONARY REACTIONS TO SILICA INHALATION

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Fracturing quartz produces radicals on the fracture planes and generates hydroxyl radicals (OH) in aqueous media. OH production is directly associated with silica-induced cell damage and phagocyte activation in vitro. This OH production in vitro is inhibited by desferol, an iron chelator. The present objective was to determine if iron increased the ability of inhaled silica to cause inflammation and lung injury. Male Fischer rats were exposed 5 hrs/day for 10 days to filtered air, 20 mg/m^3 freshly milled silica (30–40 ppm iron), or 20mg/m^3 freshly milled silica doped with iron (>500 ppm iron). High iron silica produced ≈ 70% more radicals in vitro than the low iron silica. Compared to inhalation of low iron silica, high iron silica resulted in greater neutrophil recruitment (↑900%), macrophage production of oxygen radicals measured by electron spin resonance or chemiluminescence (↑32% or 90%, respectively), nitric oxide production by macrophages (↑71%), and lipid peroxidation of lung tissue (↑38%). The results suggest that inhalation of a mixture of iron and freshly fractured silica may be more pathogenic than inhalation of silica alone.

1392 THE ROLE OF METAL-CATALYZED OXIDANTS IN LUNG INJURY AFTER PARTICLE EXPOSURE

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We tested the hypothesis that a metal initially complexed to the surface of a particle could catalyze the generation of oxidants and be associated with an inflammatory injury of the lung. Polystyrene beads (0.945 μm; Bangs Laboratories, Carmel, IN) with surface -COOH groups were treated with NaOH and then agitated in either 100 μM ferric ammonium sulfate (latex-Fe^{3+}) or sterile water (latex) for 30 minutes. *In vitro* oxidant generation was measured as the absorbance of thiobarbituric-acid-products (A_{532}) in reaction mixtures including 1.0 mM H_2O_2, 1.0 mM ascorbate, 1.0 mM deoxyribose, and particles. Incubations with 1000 μg/ml latex-Fe^{3+} were increased relative to those including either an identical mass of latex or saline (A_{532} of 1.146 ± 0.036, 0.372 ± 0.015, and 0.093 ± 0.016 respectively; mean ± standard deviation). Similarly, maximal luminol-enhanced chemiluminescence by rat alveolar macrophages (1.0 × 10^6 cells/ml) was elevated with exposures to 1000 μg/ml particles with complexed metal (3.0 ± 0.1, 0.2 ± 0.1, and 0.0 ± 0.0 V). Finally, 24 hours after intratracheal instillation into 60 day old, male Sprague Dawley rats, inflammation was significantly increased after latex-Fe^{3+} (lavage neutrophils of 37 ± 11%, 16 ± 9%, and 2 ± 3% and lavage proteins concentrations of 0.51 ± 0.05, 0.26 ± 0.05, and 0.11 ± 0.03 mg/ml). We conclude that metal initially complexed to the surface of a latex particle can be associated with increments in oxidant generation and lung injury. This abstract does not necessarily reflect EPA policy.

1393 SUSCEPTIBILITY AND DIET: THE ROLE OF VITAMIN C IN ACUTE OZONE TOXICITY IN GUINEA PIGS

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Recent evidence suggests that most Americans consume less than the optimal five servings of fruits and vegetables per day, and many have less than saturating levels of vitamin C (C) in their blood plasma. C is actively concentrated into airway lining fluids where it serves as an antioxidant to protect against oxidant pollutants. The present study investigated possible endpoints that could be used in a human study of the interaction between C and inhaled ozone (O_3). Guinea pigs were fed a C deficient chow until plasma C was ~20% of normal (2 weeks). O_3 exposure (2 hr, 1.0 and 0.4 ppm) was followed immediately by a pulmonary function test battery, and by preparation of tissues for morphological and biochemical assessment (also at 24 hr post exposure). Results showed that bronchoalveolar lavage (BAL) protein was increased by O_3 exposure and further increased by C deficiency, especially at the 0.4 ppm level. BAL neutrophil percentage and all pulmonary function measurements were altered by O_3 but not by C status. The percent denuded epithelium in the proximal alveolar region was increased from 3% in controls to 45% in O_3 (1 ppm) exposed to 62% in O_3 exposed C deficient animals. Use of labeled O_3 (oxygen-18) for exposure resulted in enhanced labeling of BAL constituents in C deficient animals, consistant with the hypothesis that C prevents O_3 from reacting with lipids and proteins of the lung lining material. *In vitro* studies confirmed that incorporation of oxygen from $^{18}O_3$ into proteins is inhibited by the presence of C in the medium. In summary, dietary C appears to alter cell injury endpoints of susceptibility to O_3 while some lung function and inflammatory indicators appear to be unaffected. (This abstract does not necessarily represent EPA policy).

1394 OZONE-INDUCED DNA-BINDING ACTIVITY OF TRANSCRIPTION FACTORS AND IL-8 PRODUCTION IN A549 CELLS

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Ozone (O_3), one of the most reactive oxidant to which humans are routinely exposed, induces inflammation in the lower airways. The airway epithelium is one of the first targets inhaled O_3 will encounter. However, its role in ozone-induced airway inflammation is not well understood. Epithelial cells could contribute to the neutrophil influx seen after exposure to O_3 by releasing interleukin-8 (IL-8), a potent neutrophil chemotactic factor. Expression of inducible genes involved in the inflammatory and immune response, such as IL-8, is controlled by transcription factors. The IL-8 gene appears to be under the control of the transcription factors NF-KB, AP-1, and NF-IL6. A human type-II-like epithelial cell line (A549) was grown to a monolayer in Costar Transwells (Costar, Cambridge, MA) and exposed *in vitro* to 0.1 ppm O_3/air. Induction of DNA-binding activity of transcription factors was assessed by mobility shift. Exposure of A549 to 0.1 ppm O_3 for 5 hours induced DNA-binding activity of NF-KB, AP-1, and NF-IL6. Additionally, the effects of O_3 on IL-8 release was measured in A549 cells 48 hours following exposure as described above. IL-8 levels in the basolateral supernatant of O_3-exposed A549 cells was significantly increased as compared to air-exposed cells. These results link ozone-induced DNA-binding activity of transcription factors and the production of IL-8 by epithelial cells, thus demonstrating a possible cellular mechanism of epithelial-mediated airway inflammation. (Sponsored by EPA R819342).

1395 PREFERENTIAL O_3 ABSORPTION SUBSTRATES: EFFECTS ON OXIDATION OF LUNG LINING LAYER LIPIDS.

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O_3 absorption occurs due to reaction within the surface lining layer (SLL) and produces oxidation of unsaturated fatty acids (UFA). However, the relationships among O_3 absorption rates, the distribution of SLL substrates, and the induction of oxidant stress remain undefined. SLL (as bronchoalveolar lavage fluid; BALF) and model systems were exposed under well-mixed, steady-state conditions (~ 0.5 ppm O_3; 25°; 20 min). Substratespecific absorption rates and UFA oxidation products (aldehydes, TBARS) were determined. **Results: a)** Ascorbate (AH_2) and egg phosphatidylcholine liposomes (EggPC) produce greater O_3 uptake than glutathione (GSH). In combined systems, AH_2 accounts for most O_3 uptake. **b)** Exposure of BALF produces O_3-specific (nonanal and heptanal) and autoxidation aldehydes (hexanal) in a dose and time-dependent manner. **c)** Addition of either BALF or AH_2 to EggPC increases O_3 uptake (AH_2 >> BALF). However, TBARS are increased by BALF but limited by AH_2. **d)** Removal of AH_2 from BALF decreases uptake but substantially increases nonanal, heptanal, and hexanal production. **Conclusions**: AH_2 and lipids are the predominant O_3 absorption substrates. AH_2 limits direct O_3 attack of UFA and quenchs O_3-induced autoxidation. However, based on the observed relationships between O_3 absorption and UFA oxidation, for a given [O_3], dose/response relationships may be complex. Due to inhomogeneities in SLL constituent profiles, increased absorption may not necessarily lead to increased oxidative events. (CIAR 90–23, HEI 91–7, NIH T32ES07254, and Meadows Foundation)

1396 EFFECTS OF ASCORBATE AND HEXAVALENT (CrVI) AND PENTAVALENT CHROMATE (Cr V) IN SEVERAL SYSTEMS, INCLUDING LUNG CELLS, AND IN VITRO

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Previous studies in our laboratories have shown that Cr(V) and reactive oxygen species (ROS) are produced in A549 cells on addition of Cr(VI), as

dichromate. (The Toxicologist 15:312). Both may contribute to oxidative stress. The current studies were designed to evaluate the stop-flow technique for kinetic studies of Cr(VI) reduction. Studies using the Olis Rapid Scanning Monochromator show that the in vitro reduction of Cr(VI) by ascorbate is at least a two step process en route to Cr(III). A synthetic Cr(V) chelate, [potassium bis (2-ethyl, 2-hydroxy-butyrato) chromate V] was reduced in vitro by ascorbate also in a two-step process, with production of an unidentified intermediate and Cr(III). Addition of 2-ethyl, 2-hydroxy butyric acid (10 mM) [as a trapping agent for CrV)] to a dichromate (50 μM)/ascorbate (2 mM) mixture did not result in production of any intermediate with maximal absorption at 355 nm [indication of Cr(V)]. Maximal reduction of dichromate at pH 7.4 was found with excess ascorbate, with a minimal ratio of ascorbate:-dichromate of 20:1. Preliminary studies with mouse lung homogenate and H4 cells using the stop-flow system suggest that this technique may be applicable to kinetic studies of dichromate reduction in these systems.

1397 SIDESTREAM CIGARETTE SMOKE EXPOSURE AND PULMONARY ANTIOXIDANT ENZYMES IN NEONATAL AND ADULT RATS

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Passive cigarette smoke exposure has been implicated in the development of respiratory disorders in humans. Cigarette smoke contains highly reactive radicals which can potentially cause oxidative damage to lung tissue. Past studies have suggested that while neonates exhibit antioxidant enzyme induction in response to oxidative stress, the adults do not. To determine if smoke exposure also elicits similar response, we compared the pulmonary antioxidant enzymes of neonatal and adult rats after exposure to sidestream cigarette smoke (SS-CS). Adult female Sprague Dawley rats were exposed 6 hrs/day to SS-CS in a whole body exposure chamber, and neonates received exposure for 2 hrs/day, beginning at age day-1. Control animals were maintained in parallel. Animals were sacrificed after 1 and 4 weeks of daily exposure and the lungs were processed for analysis of antioxidant enzymes; Mn/CuZn-superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) activity. One-week exposure to SS-CS did not produce any change in antioxidant enzyme levels of neonates or adults. Exposure to SS-CS for 4 weeks, however, significantly increased the activity of Mn-and CuZn-SOD in neonatal but not the adult rats. Other enzymes were not significantly altered in either group. These results suggest an adaptive response of neonatal but not the adult rats to oxidative stress induced by sidestream cigarette smoke exposure (supported by NIEHS ES05604).

1398 EVIDENCE OF OXIDATIVE INJURY IN RATS FOLLOWING SMOKE INHALATION INJURY

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Oxygen radical mechanisms have been implicated in the pathophysiology of smoke inhalation injury. The present study further characterizes lung, liver, heart and kidney antioxidant enzymes and indices of lipid peroxidation in a smoke inhalation injury rat model. Adult rats (n=5/gp) were exposed to cooled western bark (fir and pine) smoke (S) or air (controls, C) for 16.25 min. Groups were then euthanized at 1, 12, 24, 48 and 96 h and tissues removed. Analysis of bronchiolar lavage (BAL) fluid from S rats revealed significantly higher protein levels at 12 h and 24 h after exposure when compared with C. Lung water content was not different between the 2 groups. In S rats, of the antioxidant enzymes assayed, lung glutathione peroxidase activity was about 25% higher ($p<0.05$) than C at 12, 24 and 48 h. In contrast, both Cu, Zn and Mn-superoxide dismutase activities were significantly lower in S lungs than C at 12 h and 24 h after exposure. These data were associated with significant elevations in indices of lipid peroxidation. Concentrations of thiobarbituric acid-reactive substances were 2-fold higher and levels of conjugated dienes were 170% and 70% higher at 12 h and 24 h, respectively, in S lungs than C. These variables were not significantly different in the other organs assayed. These data suggest that in this rat model of smoke inhalation injury, evidence of oxidative injury was manifest at 12 h and 24 h after smoke exposure and appeared limited to the lung. However, increasing smoke exposure an additional 30-60 sec resulted in 100% mortality in this model.

1399 MOLECULAR DETERMINANTS OF ORGANIC DUST EXPOSURES IN RESPIRATORY CELLS

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We are utilizing a cell model to explore the pro-inflammatory responses of rat alveolar macrophages (AM) and respiratory epithelial cells to aqueous extracts of respirable grain dusts collected during routine agricultural operations. These studies are aimed at elucidating the underlying mechanisms of pulmonary injury and adaptation in agricultural workers exposed to organic dusts. Exposure to grain dusts or purified bacterial endotoxin/LPS resulted in a dose-dependent inhibition of cell growth in both AM and epithelial cell lines. Comparison of growth curves suggested the presence of other, unidentified, cytotoxic components of grain dusts, besides endotoxins. The inhibition of cell proliferation was preceded by the production of reactive oxygen species (ROS) in AM cells, measured by spectrophotometric determinations of hydrogen peroxide and superoxide anion, as well as by DNA fragmentation that is associated with apoptotic cell death, measured by DNA gel electrophoresis and ELISA. The comparative magnitudes of these pro-inflammatory responses suggest that the AM cells may be pivotal in producing toxicity in other respiratory cells following inhalation of organic dusts. We have also detected an immediate induction of the stress protein, metallothionein, in AM cells following their exposures to grain dust extracts and endotoxin. We are further studying the role of ROS in triggering apoptosis and whether metallothionein can inhibit ROS-related cell injury, as well as measuring the activation of the DNA transcription factor, NF-KB, in these cells following their exposures to organic dusts. (Supported by NIOSH #U07/CCU807121).

1400 INDUCTION OF HEPATIC MICROSOMAL ENZYMES IN THE PUERTO RICO GROUND LIZARD

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The Puerto Rico ground lizard, *Ameiva exsul*, is being evaluated as a potent sentinel model to monitor environmental contamination in Puerto Rico. Induction of hepatic microsomal enzymes is being examined as an indicator of xenobiotic exposure. Previous enzymatic studies have verified the presence of the mixed-function oxidase enzyme system and the phase II enzyme glutathione-S-transferase in the liver of *Ameiva exsul*. The purpose of this study was to evaluate the response of the hepatic cytochrome P450 enzymes to inducers like β-naphthoflavone (BNP). Lizards were captured from the field and housed in the laboratory for 21 days to allow the dissipation of any previous induction. Microsomes were prepared from lizards treated for four days with either β-naphthoflavone in corn oil or corn oil alone (controls). Total P450 spectra and P450-dependent enzyme activity were measured. P450 and B5 concentrations as well as enzyme activities in microsomes showed no significant differences except for a 5.5 - fold increase in ethoxyresorufin-o-deethylase (EROD) activity. EROD activity in control was 0.4 nmoles/min/mg compared with 2.2 nmoles/min/mg BNP treated animals. This apparent induction of CYP1A1 isozyme is supported by cross-reaction of a microsomal protein of about 50 kDa with a rabbit polyclonal anti CYP1A1. Initial studies indicate that exposure to other xenobiotics like pregnenolone-16α-carbonitrile, isoniazid, and phenobarbital can selectively induce other hepatic isozymes of cytochrome P450.

1401 EFFECT OF BITTER MELON (MOMORDICA CHARANTIA) JUICE ON THE HEPATIC CYTOCHROME P450 (CYP) ACTIVITIES IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Bitter melon (Karela fruit) has been reported to have antidiabetic and antitumor activities. In the present study we have investigated the effect of oral feeding of karela fruit juice (KJ) on liver CYP enzymes in the STZ diabetic rats. Male Wistar rats (200–250g) were made diabetic by a single I.P. dose (60 mg/Kg) of STZ. Animals were divided into 4 groups: STZ-induced diabetic, KJ fed (10 ml/kg/day for 10 wk) -diabetic, KJ alone and untreated control group. At the end of 10th week animals were sacrificed and liver microsomes prepared by ultracentrifugation. CYP contents, ethoxycoumarin -0-deethylase (ECOD), ethoxyresorufin-0-deethylase (EROD), aniline hydroxylase (AH), aminopyrene N-demethylase (APD) activities and lipid peroxida-

tion (LPO) were measured in the microsomes of all groups of animals. Diabetic rats exhibited a two fold increase in AH and EROD activities and a decrease in the activities of APD and ECOD. Feeding of KJ to the diabetic animals brought the level of enzymes close to that of control animals with the exception of ECOD which was further reduced to 60% of control value. CYP and protein contents remained unchanged both in the diabetic and KJ fed animals. LPO was drastically reduced (2–5% of the control value) both in diabetic and KJ fed groups. Western immunoblot analysis showed a differential expression of CYP isoenzymes in diabetic rats and the KJ feeding reduced the increased expression of these CYP isoenzymes. Our results suggest that the alterations in hepatic drug metabolizing activities of the diabetic animals may be related to the altered expression of different CYP isoenzymes. In addition KJ appears to have insulin like action in diabetic animals.

1402 DRUG METABOLIZING ENZYME INDUCTION BY HETEROCYCLIC NAPHTHALENE AND PHENANTHRENE DERIVATIVES DIFFERS IN THE ADOLESCENT AND ADULT MALE RAT

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Studies with nitrogen heterocycles in adult male rats have shown that structurally similar compounds can have very different effects on hepatic drug metabolizing enzymes. The present study demonstrates that the induction profile for a single compound may vary with the maturity of the rat. Adult (180–250g) and adolescent (80–100g) male rats were treated with naphthalene, quinoline (Q), isoquinoline (IQ), 3,4- (3,4-BQ), 5,6- (5,6-BQ), or 7,8- (7,8-BQ) benzoquinoline at 75mg/kg, i.g. daily for 3 days. Naphthalene did not affect any enzymes. 7,8-BQ showed the largest maturity-dependent difference in the induction response. In adult rats, cytochrome P450 concentration, 7-ethoxyresorufin deethylation (EROD), UDP-glucuronosyltransferase (UGT) activity towards 1-naphthol, 4-nitrophenol, and morphine, glutathione S-transferase (GST), NAD(P)H quinone oxidoreductase (QOR), and microsomal epoxide hydrolase (mEH) activities were all elevated. In adolescent rats, 7,8-BQ did not change P450 concentration and elicited only a minor increase in EROD activity. Increases in UGT(1-naphthol), UGT(4-nitrophenol) and QOR activities were diminished slightly in magnitude. Furthermore, increases in UGT (morphine), and GST and mEH activities were not significant. In contrast, 5,6-BQ while eliciting no response in adult rats caused increases in UGT(morphine) and also in GST and QOR activities in adolescents. 3,4-BQ treatment elicited similar responses in UGTs and mEH, but increased QOR and decreased GST responses in adolescents as compared to adult. Q and IQ responses were of higher magnitude in adolescents compared to adult, especially for GST, QOR and mEH. With the diminished P450 induction response in adolescent rat, 7,8-BQ would almost appear to be a monofunctional (Phase II selective) inducer, as compared to the bifunctional nature seen in adult. Despite an increased induction response with Q and IQ, they remained monofunctional (Phase II selective) in nature.

1403 THE EFFECTS OF m-XYLENE ON RAT CYP2B1 AND CYP1A1 IN LIVER, LUNG, AND NASAL MUCOSA

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Previous studies showed that *m*-xylene (*m*-XYL) selectively inhibited CYP2B1 and CYP1A1 activities in lung but not in liver. The aldehyde metabolite of *m*-XYL is thought to be, at least in part, responsible for this inhibition and can be quantitated in rat tissues. The purpose of this study was to determine the effects of *in vivo* administration of *m*-XYL on CYP2B1 and CYP1A1 activities, and to quantitate *m*-XYL and *m*-tolualdehyde (*m*-ALD) levels in tissues. In the present study rats were given *m*-XYL (1.0 g/kg, ip) for 1, 3, or 6h. Liver, lung, and nasal mucosa CYP2B1 and CYP1A1 activities were measured using benzyloxy- and ethoxyresorufin (BROD or EROD), respectively. Blood *m*-XYL levels were quantitated using gas chromatography. Tissue levels of *m*-ALD were measured by adduct formation with 2,4-dinitrophenylhydrazine and quantitated by HPLC. Blood *m*-XYL levels increased steadily between 1h and 6h. CYP2B1 activity was inhibited in lung at 1h (99%), 3h (53%), and 6h (30%) as compared with controls. Pulmonary CYP1A1 activities showed a steady decline from control at 1h (80%), 3h (70%), and 6h (64%). Hepatic CYP2B1 showed no change at 1h and 3h, but increased to 340% of controls at 6h. CYP1A1 activity in the liver was unchanged at all time points. Nasal mucosa CYP2B1 and CYP1A1 activities were inhibited at all time points. Tissue levels of *m*-ALD were shown to be the highest in nasal mucosa at 1h following *m*-XYL exposure. This data further supports the notion that metabolites of *m*-XYL may be involved in the observed isozyme effects.

1404 CATALYSIS OF CYSTEINE CONJUGATION (CYSCON) AND PROTEIN BINDING (PROT-BNDG) OF ACETAMINOPHEN (ACET) BY MICROSOMES FROM LYMPHOBLASTS TRANSFECTED WITH cDNAs OF HUMAN CYPs

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We have previously found that the hepatic, microsomal **CYS-CON** and **PROT-BNDG** of **ACET** have different kinetics and that the microsomal, **PROT-BNDG** is only to intralumenal proteins. Together these data suggested that **ACET** oxidative metabolism forms two pools of the reactive metabolite, N-acetyl-*p*-benzoquinone imine: one on the cytosolic surface and the other within the microsomal lumen. Hence, some CYPs should have their active site on the cytosolic surface and others within the lumen. We examined this question by comparing the rates of **CYSCON** and **PROT-BNDG** by microsomes from lymphoblasts tranfected with cDNAs for human CYPs. We found that CYP2D6 catalyzed only **CYS-CON**; CYP1A2 and 3A4 only **PROT-BNDG**; CYP2E1 both; and CYP1A1, 2A6 and 2B6 neither. These data suggest that CYP2D6 has its active site on the cytosolic side; CYP1A2 and CYP3A4 on the lumenal side; and CYP2E1 on both. In mouse studies we found that ethanol administration increased **PROT-BNDG** by 365% and **CYS-CON** by 161%. CYP2E1 and 2B increased while 3A decreased. CYP1A1, 1A2 and 2A6 did not change. These data indicate that in control mice, CYP2E1 catalyzes 83% of **PROT-BNDG** and 44% of **CYS-CON**. **CYP2D** catalyzes the remaining 56% of the **CYS-CON**.

1405 ENHANCED CYTOCHROME P450 METABOLIC-INTERMEDIATE COMPLEX FORMATION FROM METHYLENEDIOXYBENZENE DERIVATIVES IN CYP3A ENRICHED RAT LIVER MICROSOMES

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Following recent reports of enhanced rates of amine cytochrome P450 metabolic-intermediate (MI) complex formation in microsomes from dexamethasone pretreated rats and rifampicin pretreated rabbits, the role of increased CYP 3A in MI complex formation from non-nitrogenous methylenedioxybenzene derivatives and from N-benzyl-1-aminobenzotriazole (BBT) was examined. Rats were pretreated with phenobarbital or dexamethasone at two doses for four days and in vitro rates of MI complex formation in subsequently isolated hepatic microsomes were monitored by NADPH dependent absorbance changes at 455 nm in the presence of saturating substrate. Phenobarbital induction, while producing major changes in pentoxyresorufin dealkylase activity, produced only minor increases in rates of MI complex formation from piperonyl butoxide, safrole and isosafrole, and somewhat larger increases from methylenedioxybenzene and BBT. No phenobarbital-elicited changes approached the magnitude of those seen with dexamethasone induction. Correlation coefficients (r) between CYP 3A-selective MI complex formation from troleandomycin and MI complex formation from piperonyl butoxide, safrole, isosafrole and methylenedioxybenzene were 0.90, 0.85, 0.92 and 0.87. For BBT the correlation coefficient was 0.88. For comparison, the correlation coefficient between two CYP 3A-selective reactions, erythromycin demethylation and troleandomycin MI complex formation, was 0.97. Thus, CYP 3A appears to be the major contributor to high rates of MI complex formation from certain methylenedioxybenzene derivatives in rat hepatic microsomes. CYP 2B induction also plays a role in enhanced MI complex formation from methylenedioxybenzene itself and BBT.

1406 STRUCTURE-ACTIVITY RELATIONSHIPS FOR INDUCTION AND INACTIVATION OF CYTOCHROME P-450 2B ENZYMES (Cyp2b) BY SYNTHETIC NITRO MUSKS

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Musk xylol (MX; 1,3,5-trinitro-2-*t*-butylxylene) induces and inhibits mouse Cyp2b enzymes. An amine metabolite, formed by nitro reduction in intestinal

flora, produces mechanism-based inactivation of the enzyme. Because other nitro musks, including musk ketone (MK; 3, 5-dinitro-2, 6-dimethyl-*t*-butylacetophenone) and musk tibetene (MT; 2, 6-dinitro-3,4,5-trimethyl-*t*-butylbenzene), lack the nitro group substitution required for enzyme inactivation, it is likely that these musks would induce, but not inactivate, Cyp2b. To test this hypothesis, male B6C3F1 mice were dosed orally with MX, MK and MT at dosages of 0.03 and 0.67 mmol/kg (approximately 10 and 200 mg/kg) for 7 days. For all three chemicals, the low dose had no effect on liver weight or microsomal enzyme activity. At the high dosage, MX increased liver weight by about 50%, whereas MK and MT increased liver weight by 20%. MX, MK and MT increased Cyp2b protein levels, with MX > MK > MT. Although MX treatment increased Cyp2b protein levels, there was no associated increase in Cyp2b enzyme activity, determined by pentoxyresorufin-O-dealkylase (PROD) activity. In contrast, MK and MT increased PROD about 6- and 3-fold, respectively, consistent with the increase in Cyp2b protein levels. These results confirm that, although not as potent as MX, MK and MT induce Cyp2b. Moreover, because the nitro function of MX required to produce enzyme inactivation is not present in MK and MT, neither MK or MT inactivate Cyp2b.

1407 GENETIC ANALYSIS OF SELECTED P450 AND PHASE II ENZYMES IN TWINS EXPOSED TO ENVIRONMENTAL TOBACCO SMOKE

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Variation in the inducibility of P450 enzymes such as CYP1A2 and Phase II enzymes such as Nacetyltransferase-2 (NAT2) by polycyclic hydrocarbons and nitrosamines in different individuals is thought to be related to the risk of developing smoking related cancers. However, genetic analysis of these enzymes has been impeded by the lack of reliable *in vivo* assays of these enzymes' activities. Kalow and Tang devised a method to estimated the indices of enzyme activity for CYP1A2, NAT2, xanthine oxidase (XO), and CYP2E1. Their method utilizes the ratios of several urinary caffeine metabolites. To determine the components of variation in CYP1A2 and NAT2 activity, we measured the urinary caffeine metabolites from two sets of adolescent twins. One group included 20 pairs with one or more parent that smoked (passive smokers); the second group of 20 pairs had parents that did not smoke (non-smokers). Each twin was asked to drink a can of Coca-Cola at bedtime and their first morning urine was collected. The relative amounts of 10 caffeine metabolites were measured by HPLC. The 10 x 10 metabolic covariance matrices of the passive smokers and non-smokers showed a highly significant difference (X^2 = 127, 45df, $p<0.005$). In an overall genetic analysis of indices of activity for individual enzymes, the major component of variation in CYP1A2 activity was due to shared environmental effects, while the major component of variation in NAT2 activity was due to genetic effects. To test the hypothesis that exposure to cigarette smoke was the environmental factor detected in the overall analysis of CYP1A2, we compared the results for the passive smoking twins with the non-smoking twins. Evidence for genetic variation was found only in the non-smoking twins while environmental factors were the only source of covariation detected in the passive smoking twins. Sponsered in part by NIH RO1HD30252-01A2.

1408 INDUCTION OF MURINE CYTOCHROME P450-2E1 BY ETHANOL ADMINISTEREd IN THE LEIBER-DECARLI® LIQUID DIET

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Acute administration of ethanol is known to potently induce hepatic cytochrome P450-2E1. The 2E1 isozyme is important to environmental toxicology because this isozyme initiates the metabolism of numerous toxins to which humans are exposed. For example, benzene, a common pollutant and bone marrow toxin, is a substrate for the 2E1 isozyme in the liver, the primary site of benzene metabolism, and in the bone marrow, the target site of benzene toxicity. Although the effect of acute, bolus ethanol treatment on 2E1 is well characterized, less is known about responses to repeated dietary exposure to moderate levels of ethanol, which more closely mimics daily human consumption of ethanol. The objective of this pilot study was to determine the effects of moderate ethanol exposure in a liquid diet on the 2E1 levels in the liver. We maintained female CD-1 mice on the Leiber-DeCarli® liquid diet containing various levels of ethanol (1.5%, 2.5% and 3.5% v/v) for up to two weeks. We found that consumption of the 2.5% ethanol diet for two weeks induced 2E1 activity by 75%, while the 1.5% diet did not affect 2E1 activity. Consumption of the 3.5% ethanol diet for one week caused a 3-fold increase in 2E1 activity without any apparent immuno- or hepatotoxicity as assessed by organ weights and histology. Also, in contrast to a previous report, Western blots for 2E1 in murine bone marrow showed a band which migrated to about 52 kd, suggesting constitutive expression of 2E1 in the bone marrow. The 3.5% ethanol liquid diet appears to be a useful system for studies investigating the effect of ethanol on the toxicity of chronic xenobiotic exposure. Supported by: NIEHS Training Grant-T35ES07294 and the Meadows Foundation.

1409 DOSE-RESPONSE RELATIONSHIPS FOR CYTOCHROME P450 INDUCTION BY PHENOBARBITAL IN THE COTTON RAT (SIGMODON HISPIDUS)

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The dose-dependent induction of hepatic CYP2B and CYP3A in male Cotton rats was determined following dietary exposure (14 days) to 0–1500 ppm phenobarbital. Induction was measured using catalytic and immunospecific endpoints. Maximal CYP2B induction, as measured by pentoxy- (PTR) or benzyloxyresorufin (BZR) *O*-dealkylation or testosterone 16β-hydroxylation, was less than five-fold, compared to the controls. Microsomal protein immunoreactive with polyclonal anti-rat CYP2B1 was induced to a greater extent by phenobarbital. This increase was paralleled by induction (up to 18-fold) of 7-ethoxy-4-trifluoromethylcoumarin (7-EFC) *O*-deethylation activity. CYP3A was induced less than 2-fold, as measured by testosterone 6β-hydroxylation or immunoreactivity with polyclonal anti-rat CYP3A1. This induction of a protein immunoreactive to anti-rat CYP2B1 that demonstrates increased 7-EFC *O*-deethylation activity but minimal effects on PTR- or BZR *O*-dealkylation or testosterone 16β-hydroxylation represents a clear species difference in the substrate specificity of the phenobarbital-inducible hepatic cytochrome(s) P450, compared to *Rattus norvegicus*.

1410 ARYL HYDORCARBON RECEPTOR (AhR) FUNCTION IN BASAL AND DIFFERENTIATING MURINE KERATINOCYTES

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*Cyp*1a-1 induction in murine epidermis occurs following exposure to planar aromatic hydrocarbons (PAHs) and dioxins (i.e., 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin; TCDD). This induction is mediated by the interaction of PAHs/TCDD with the aryl hydrocarbon receptor (AhR). We have examined AhR function and localization in both basal and differentiating murine epidermal keratinocytes maintained in a chemically-defined, serum free medium (SPRD-111). Following exposure to TCDD, inducible 7-ethoxyresorufin O-deethylase activity (EROD; an indicator of CYP1A1 activity) is observed primarily in cells undergoing terminal differentiation. Immunofluorescence microscopy indicates that the AhR and aryl hydrocarbon receptor translocator (ARNT) proteins are localized to the nucleus of both basal and differentiating keratinocytes. Nuclear localization of these proteins is independent of prior exposure to TCDD. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of CYP1A1 heterogeneous nuclear RNAs (hnRNA) indicates that AhR-dependent transcriptional activation of *Cyp*1a-1 preferentially occurs in differentiating keratinocytes. These results suggest that the transcriptional activation of *Cyp*1a-1 by the AhR complex is dependent on keratinocyte commitment to terminal differentiation. (Supported by NIH grant CA34469).

1411 EVALUATION OF CHEMICALS AS INHIBITORS OF TROUT P450s

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This study examined the ability of 27 mammalian P450 inhibitors to inhibit P450-mediated monooxygenases in liver microsomes from β-naphthoflavone-treated rainbow trout. The CYP1A inhibitors, α-naphthoflavone and ellipticine, strongly inhibited CYP1A-mediated dimethylbenz[a]anthracene (DMBA) hydroxylase activity and CYP2K1-mediated lauric acid (ω-1)-hydroxylase (LAOH) activity. The global inhibitors, metyrapone and chloramphenicol, had no effect on these enzyme activities. Triacetyloleandomycin,

a CYP3A inhibitor, had no inhibitory effect on progesterone 6β-hydroxylase (PROH) activity catalyzed by P450 LMC5, a CYP3A-like enzyme in trout. Gestodene, another CYP3A inhibitor, strongly inhibited PR-OH activity and, unexpectedly, ethoxyresorufin-O-deethylase (EROD) activity. 17α-Ethynylestradiol and 5, 8, 11, 14-eicosatetraynoic acid also were highly inhibitory toward PR-OH. Ketoconazole and miconazole were potent inhibitors of all the measured P450 activities. However, none of the above enzyme activities was selectively inhibited by any of the chemicals used. (Supported by NIH Grants ES00210, ES03850, ES04766 and ES05533).

1412 EFFECT OF XENOESTROGEN EXPOSURE ON THE EXPRESSION OF CYTOCHROME P450 ISOFORMS IN RAINBOW TROUT LIVER

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Injection of juvenile rainbow trout with 17β-estradiol represses expression of several cytochrome P450s and reduces hepatic lauric acid hydroxylase (LA-OH) activity. However, there is no information about the role of environmental estrogens such as pesticides, phytoestrogens and surfactants in the regulation of trout P450s. In this study, therefore, four putative estrogenic chemicals: methoxychlor (20mg/kg), diethylstilbestrol (15 mg/kg), 4-(*tert*-octyl) phenol (25 and 50 mg/kg) and biochanin A (25 and 50 mg/kg) were injected (ip) on days 1, 4 and 7 into one-year-old juvenile trout and the fish then sacrificed on day 9. Plasma vitellogenin was used as a biomarker for estrogenicity and all of the test chemicals caused increases in plasma vitellogenin levels. The xenoestrogens all decreased LA-OH activity and, as shown by Western blots, also markedly repressed expression of hepatic CYP2K1, CYP2M1 and P450 LMC5. However, both doses of biochanin A also induced CYP1A levels and greatly increased hepatic EROD activity. Northern blot results indicated that CYP2K1 mRNA also was repressed in the four trout groups. These findings suggest that methoxychlor, diethylstilbestrol, 4-(*tert*-octyl)phenol and biochanin A are all estrogenic and act like 17β-estradiol in repressing the expression of several P450 isoforms in the trout liver. Biochanin A, however, also was found to induce CYP1A1 in this fish species. (Supported by NIH Grants No. ES000201, ES03850, ES04766, and ES05533).

1413 HETEROLYTIC O-O BOND CLEAVAGE OF HYDROPEROXIDES BY MUTANT CYTOCHROME P450 2F3 EXPRESSED IN E. COLI

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Cytochrome P450 *2F3* cDNA containing 1473 bp in the transcribing region and encoding a polypeptide of 491 amino acids was cloned from a goat lung cDNA library. The predicted amino acid sequence of 2F3 possessed 82% similarity to human CYP2F1 and mouse Cyp2f-2. Northern blot analysis with *2F3* of goat lung and liver mRNA revealed that a 2.0 kb transcript was detected in a highly tissue-specific manner only in lung but not in liver. This cDNA clone was mutated within the first nine codons, which favors expression in *E. coli*, and placed in pTrc 99A expression vector and then used to transform *E. coli* XL1-Blue cells. The expressed P450 content was 20-91 nmol per liter of culture and the protein was characterized by western immunoblotting using a rabbit anti-mouse 2f-2 polyclonal antibody. Cytochrome P450s have been shown to catalyze the cleavage of organic hydroperoxides by both heterolytic and homolytic processes, and this can be used to characterize the active site of the enzymes. Expressed CYP2F3 was incubated with 2,6-di-*tert*-butyl-4-hydroperoxy-4-methyl-2,5-cyclohexadienone (BHTOOH) or 4-hydroperoxy-2,4,6-trimethyl-2,5-cyclohexadienone (TMPOOH). Ratios of heterolysis to homolysis were determined by HPLC analysis. The expressed enzyme primarily catalyzed the heterolysis of TMPOOH to produce an epoxide, demonstrating unique catalytic preference for epoxidation rather than hydroxylation. However, with the bulkier hydroperoxide, BHTOOH, 2F3-catalyzed homolysis was a principle pathway. Therefore, expressed CYP2F3 is an active enzyme that predominately catalyzes the heterolytic cleavage of TMPOOH to form the epoxide product. (Supported by USPHS grants HL13645 from NHLBI and CA41248 from NCI).

1414 REGULATORY CONTROL OF CYTOCHROME P450 1B1 IN MOUSE EMBRYO FIBROBLASTS

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Cytochrome P450 1B1 (CYP1B1) exhibits 40% sequence identity to CYP1A1 but greater differences in mRNA and genomic characteristics. CYP1B1 forms an exceptionally long 5.2 kb mRNA which is transcribed from a very compact gene that has only two introns. CYP1B1 and CYP1A1 are both regulated by the Ah receptor, and are active in the metabolism of polycyclic aromatic hydrocarbons, but with large differences in product formation. CYP1B1 expression is however enhanced by cAMP in steroidogenic tissues and is selective for stromal fibroblasts relative to epithelial cells. Upstream regions of the mouse and rat CYP1B1 genes have been sequenced and various segments have been linked to luciferase expression vector (CYP1B1-LUC). These genes do not have a TATA box but show SP-1 elements in a region immediately upstream of positions reached by primer extension of CYP1B1 mRNA. Several XRE sequences have been identified in this upstream region and further sequence analysis is in progress. Transient transfections of CYP1B1-LUC (exon 1 + 0.9kb. 5' flanking region) into mouse C3H10T1/2 embryo fibroblasts or mouse HEPA 1 hepatoma cells each showed basal expression of luciferase and 8-fold stimulation by TCDD, thus failing to reflect the more than 100-fold preference of CYP1B1 for 10T1/2 cells. An analogous CYP1A1 construct showed a 10-fold preference for TCDD-induction in HEPA cells. While this parallels the selectivity for CYP1A1 expression AhR levels are also far higher in HEPA cells. We conclude that these transiently transfected constructs poorly reproduce cell-selectivity of CYP1B1/CYP1A1 expression. The effect of full genomic incorporation of CYP1B1-LUC sequences will be reported. Funded by NIH grant CA16265.

1415 CHARACTERIZATION OF CYTOCHROME P450, FORM-SPECIFIC ACTIVITIES AND EXPRESSION IN MALE AND FEMALE ob/ob MICE

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The cytochrome P450-dependent monooxygenase system is responsible for the majority of oxidation reactions of drugs and other xenobiotics. Variations in these biotransformation activities may alter the bioavailability or efficacy of drugs, provide protection from certain xenobiotic agents, or increase toxicity of others. A mutation in the obese gene in the C57BL/6J mouse results in the mutant obese mouse (*ob/ob*), which displays several altered physiological conditions. The present research reports on the hepatic microsomal cytochrome P450-dependent activities, glutathione transferase activity, and expression in liver of selected P450 mRNAs in the *ob/ob* mouse at 3–4 months (young) and 7–8 months (old) of age. CYP2E1- and CYP3A-dependent activities were measured by p-nitrophenol hydroxylase and erythromycin demethylase, respectively. Activities for CYP1A1/1A2, CYP3A and CYP2B were estimated by determining O-deakylation of ethoxy-, benzoxy- and pentoxy-resorufin, respectively. Female mice, regardless of age, showed an obesity enhanced level of CYP2E1-dependent activity and protein as shown by Western blots. The mRNA level of CYP2E1 in female mice did exhibit age- and obesity-influenced differences in expression. No significant differences in CYP2E1-dependent activity or expression were observed in male mice. CYP3A-dependent activity was significantly higher in young males, regardless of phenotype. Benzoxy-and pentoxy-resorufin dealkylase activities did not differ among any animals; however, ethoxy-resorufin dealkylase activity, while depressed in obese animals of both sexes, were significantly different in old animals. Glutathione transferase activity was lowered in obese male mice, whereas no difference was reported between lean and obese females. **(ALR supported by NIEHS Training Grant 5 T32ES07266).**

1416 REGULATION OF CYTOCHROME P450 (CYP) EXPRESSION BY KETONE BODIES IN PRIMARY CULTURED RAT HEPATOCYTES

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Fasting, diabetes and high-fat diet have been shown to increase expression of CYP2E1, CYP2B and CYP4A, and these changes have been correlated with increased levels of circulating ketone bodies. We examined the effects of physiological levels of ketone bodies on P450 mRNA levels in primary

cultured rat hepatocytes using Northern blot technique. We found that treatment with 5 mM D-(-)-3-hydroxybutyrate (DHB), L-(+)-3-hydroxybutyrate (LHB) or acetoacetate (AA) increased CYP2B mRNA levels by ~3-, 4- and 2-fold, relative to levels in untreated cells, but were without effect on CYP2E1 or CYP4A mRNA levels. Treatment with 1 mM decanoate increased CYP2B and CYP4A mRNA levels ~3-fold. Treatment (24 hr) with 0.1 mM ciprofibrate (CIPRO), a peroxisome proliferator, increased CYP2E1, CYP2B and CYP4A mRNA levels 2-, 3-and 11-fold. Treatment of LHB or DHB in combination with CIPRO produced a synergistic increase in CYP2B levels, but did not increase CYP4A or CYP2E1 mRNA levels above those observed for CIPRO alone. These results suggest that CYP2B expression, but not CYP2E1 or CYP4A expression, is positively regulated by ketone bodies. Supported by NIH grants ES03656 and ES5657 from the National Institutes of Environmental Health Sciences.

1417 *IN VITRO* INHIBITION OF HEPATIC AND RENAL CYTOCHROME P450 3A ISOFORM BY CYCLOSPORINS A AND G

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Cyclosporin A (CsA) and G (CsG), a promising analog, are immunosuppressant drugs known to be extensively metabolized by liver cytochrome P450 enzymes particularly isoform 3A. Therapeutic use of CsA has been associated with hepatic and renal toxicity. The aim of this study was to compare in an *in vitro* microsome model the effects of CsA and CsG on hepatic and renal CYP3A and related enzyme activities. Hepatic and renal microsomes obtained from dexamethasone-pretreated Wistar rats were incubated for 60 mn with various concentrations of CsA or CsG (hepatic : 25 to 200 μM; renal : 1 to 50 μM). CYP3A was assessed by SDS-PAGE. Related enzyme activities, ethylmorphine and erythromycine N-demethylases, were determined according to Davies *et al.* (1968). The results showed a dose-related inhibition of hepatic and renal CYP3A with both CsA and CsG. Inhibitions at the maximum used concentrations were : about 40% in hepatic microsomes for CsA or CsG (200 μM), about 60% in renal microsomes for CsA or CsG (50 μM). A dose-related inhibition was also observed with hepatic and renal N-demethylase activities. At the maximum used concentrations, inhibitions were more important with CsA than CsG : up to 30% for CsA and up to 20% for CsG on hepatic as well as renal microsomes. This study demonstrates that CsG as well as CsA inhibits CYP3A and related enzymes. However, CsG seems to be less toxic when considering N-demethylase activities inhibition. With both drugs, renal toxicity is more obvious than hepatic toxicity.

1418 SITE-SPECIFIC INDUCTION OF CYP 1A1 IN DEVELOPING RAT LUNG BY ENVIRONMENTAL TOBACCO SMOKE (ETS)

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The cytochrome P450 monooxygenase system is critical in the biotransformation of a number of environmental toxicants, many of which are present in ETS. CYP 1A1 is one of the most important isoforms which metabolize components of ETS. Although it has been demonstrated that exposure to ETS at the high ambient level significantly alters the expression of CYP 1A1 and its enzyme activity in developing rat lungs compared to age-matched controls, little information is available regarding the response to ETS within a concentration range relevant to actual indoor levels. The aim of this study is to investigate the effects of exposure to low doses of ETS on the induction of CYP 1A1 in the developing rat lung and to determine whether the induction response is related to dose of exposure and to specific subcompartments of the lung. Male Sprague Dawley rats were exposed to ETS at 0, 100, 300, 500, and 1,000 $\mu g/m^3$ from birth to 120 days of age. Airway microdissection was used to determine CYP 1A1 activity in specific compartments in the lung, i.e. trachea, parenchyma and airways. CYP 1A1 isozyme activity was found to be highest in the parenchyma, intermediate in the airways and lowest in the trachea for all exposure groups and controls. There was a significant concentration dependent induction of CYP 1A1 in the airways of animals exposed to ETS beginning at 300$\mu g/m^3$, and in parenchyma beginning at 500 $\mu g/m^3$. There was no statistically significant difference in 1A1 activity in the trachea at any exposure level. Expression of CYP 1A1 protein detected by immunoblotting and immunocytochemistry demonstrated similar dose response patterns as noted in activity levels. We conclude that lung CYP 1A1 can be induced by ETS exposure at environmentally relevant levels and that the induction is dose-dependent and site-specific. (Supported by grants from the Center for Indoor Air Research and California Tobacco-Related Disease Research Program.)

1419 IMMUNOHISTOCHEMICAL QUANTITATION OF CYTOCHROME P450 AS AN INDICATOR OF HYDROCARBON EXPOSURE IN RED DRUM (SCIAENOPS OCELLATUS)

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The current determination of hydrocarbon residues in tissues is a tedious and expensive process. The development of rapid and sensitive methods for determination of hydrocarbon contamination is crucial to assessing the biological impact of these pollutants. Available data support the proposed association between increased hydrocarbon levels, induced P450 levels, and physiological dysfunction in animals, suggesting that hydrocarbon induction of P450 enzyme systems may serve as an early warning indicator of the systemic presence of hydrocarbon pollutants. We describe the development of an immunofluorescence-based procedure capable of reporting exposure to some families of hydrocarbons in the marine environment via detection of CYP1A in liver sections. Wild caught red drum were dosed by IP injection with 3-methylcholanthrene (MC) in corn oil and total microsomal P450 protein, CYP1A content, and ethoxyresorufin-O-deethylase (EROD) activity were measured. In addition, immunohistochemical quantitation of CYP1A in liver sections was performed using a fluorogenic phosphatase substrate and high resolution digital imaging. Data generated show a dose-dependent increase in total microsomal P450, CYP1A, and EROD activity in red drum following MC treatment which correlates well with immunohistochemical findings. These preliminary results indicate the validity of immunohistochemical techniques for detecting and quantifying hydrocarbon exposure.
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1420 LOCALIZATION OF CYP1B1 EXPRESSION IN NORMAL AND AH RECEPTOR KNOCK-OUT MICE

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A known teratogen, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is linked to embryonic cardiac and palate abnormalities. Cytochrome P450 1B1 (CYP1B1), a TCDD inducible form recently cloned in this laboratory, bioactivates polycyclic aromatic hydrocarbons (PAH) to mutagens. CYP1B1 is the dominant P450 expressed in primary mouse embryo fibroblasts, and exhibits a 41% identity with CYP1A1, the major PAH metabolizing cytochrome P450 from most adult tissues. In 9.5 and 10.5 day post coitus (d.p.c.) mouse embryos, CYP1B1 exhibits 5-fold higher constitutive level of mRNA expression than CYP1A1. Immunohistochemical localization in 9.5 to 12.5 d.p.c. embryos reveals specific localization of CYP1B1 to the heart, which disappeared in 16.5 d.p.c. embryos. Ah receptor (AhR) and ARNT proteins which mediate TCDD responsiveness are also expressed in the embryonic heart. Following exposure to TCDD in culture, CYP1B1 and CYP1A1 mRNA expression increased 2- and 10-fold, respectively. Additionally, CYP1B1 localizes to the urogenital crest, the apical endoderm of the limbs and mandible, and in cephalic mesenchyme all of which are consistent with our observations that CYP1B1 is predominantly expressed in undifferentiated cells of mesodermal origin. These observation are consistant with a physiological role for CYP1B1 in normal development and raise concerns that AhR responsive, CYP1B1 mediated bioactivation of PAHs may induce abnormal development. We describe the time course and tissue specificity of constitutive CYP1B1 expression in the mouse embryo. Then characterize TCDD and PAH induced alterations. Mice lacking a functional AhR gene are used to examine the role of the AhR in regulating constitutive CYP1B1 expression. NIH grant # 16265

1421 ENDOCRINE FACTORS REGULATING PHENOBARBITAL-MEDIATED INDUCTION OF CYTOCHROME P450 ARE AFFECTED BY DIETARY FAT MODULATION AND FEEDING STATUS

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We have recently identified a polymorphism in the phenobarbital (PB) - mediated expression of five forms of cytochrome P450 (CYP2B1, CYP2B2, CYP3A1, CYP2A1 and CYP2C6) and epoxide hydrolase in inbred Fischer

(F344) and Wistar Furth (WF) rat strains. These gender-specific differences resulted from supressed induction of P450 in female WF rats, and were alleviated by hypophysectomy or methimazole treatment which partially depletes thyroid and growth hormones. We further characterized the polymorphism of CYP2B1, CYP2B2 and CYP3A1 with respect to dietary fat content and feeding status through Western and Northern blot analysis. Females of the two strains were fed the following modified TEKLAD diets: Low Fat Diet (LF), Polyunsaturated Fatty Acid Diet (PUFA) or Saturated Fatty Acid Diet (SFA). PB-mediated P450 induction in F344 rats was not significantly affected by any of the diets. WF on LF had reduced induction, PUFA or SFA treatment increased levels 3-3.5-fold, and methimazole treatment of WF on LF led to a 5-fold increase in induction. F344 and WF rats on standard diet were also starved (S) or not (NS) 24 hrs. prior to sacrifice. NS F344 rats showed a 25% lower induction compared to S F344, whereas NS WF rats had 50% lower induction compared to S WF animals. Methimazole treatment of NS WF resulted again in recuperation of induction. We conclude that strain selective GH/T3 supression of PB-induction in WF rats is dependent on dietary fat. Starvation also stimulates PB-induction, but by a distinct thyroid-dependent mechanism that affects both strains.

1422 EXPRESSION OF CYP1B1 AND CYP1A1 IN C3H 10T1/2 X HEPA1C1C7 SOMATIC FUSION CELLS

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The PAH inducible P450s cyp1a1 and cyp1b1 are expressed in a variety of tissues, with CYP1a1 being preferentially induced in tissues and cell lines of epithelial origin whereas CYP1B1 is largely restricted to tissues and cell lines of mesenchymal origin. In order to investigate the regulatory mechanisms which contribute to the tissue specificity, we have generated somatic fusions of Hepa1 C1C7 cells, which only express Cyp1a1, and C3H10T1/2 fibroblasts, which only express cyp1B1. Thirteen fusion cell lines were isolated. Upon induction with TCDD all fusion cell lines expressed both, CYP1A1 as well as CYP 1B1 protein. However, all except one of the fusion cell lines expressed CYP1A1 and CYP1B1 at lower level than the corresponding parental cell line. The expression level of CYP1A1 ranged from 2.1 to 72% of CYP1A1 in Hepa cells and CYP1B1 was expressed at 1-90% of CYP1B1 in C3H10T1/2 fibroblasts. In five cell lines analyzed in greater detail, the RNA level corresponded to the observed amount of CYP1A1 and CYP1B1 protein, indicating that the stability of both protein species is unaffected by the cellular environment of the fusion cells and that the expression is regulated at the RNA level. DMBA metabolized by microsomes from TCDD induced fusion cells displayed a metabolic profile consistent with the relative expression level of CYP1A1 and CYP1B1 in each of the fusion cells. This suggests that most of the CYP1A1 and CYP1B1 protein produced in fusion cells is also metabolically active. All fusion clones expressed oxidoreductase to the same level as observed in Hepa1 cells, accounting for a general increase in CYP1B1 DMBA activities in fusion cells. Although the mechanism of transcriptional silencing of the CYP1a1 and CYP1b1 is not known, we conclude that at least the lack of TCDD-inducible CYP1a1 expression in C3H10T1/2 cells appears not to be due to tissue specific extinction, since CYP1A1 was expressed in all TCDD induced fusion cell lines, whereas expression of the liver specific marker fetal albumin was extinguished in all investigated fusion cell lines.

1423 CHARACTERIZATION OF HEPATIC CYTOCHROME P-450 ACTIVITIES IN RATS FOLLOWING INHALATION EXPOSURE TO P-CHLOROBENZOTRIFLUORIDE (PCBTF)

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PCBTF, an intermediate in the synthesis of some herbicides, is currently being evaluated as an industrial solvent. The neurotoxicity of PCBTF was evaluated in a 90-day inhalation study in male and female Sprague-Dawley rats exposed for 6 hours/day to 0, 10, 50 and 250 ppm PCBTF. Following exposure, the livers were weighed, frozen and microsomal fractions prepared to assess cytochrome P-450 (CYP-450) content and activities. A significant increase in liver weight and CYP-450 content in female rats was observed in the high dose group (250 ppm), while no significant changes were observed in males. 7-Ethoxyresorufin-o-deethylase (EROD), a measure of CYP1A1 activity, and acetanilide and phenacetin metabolism, a measure of CYP1A2 activity, were increased about two fold in males and females at the high dose (250 ppm). Pentoxyresorufin-o-deethylase (PROD) and benzyl-oxyresorufin-o-deethylase (BROD), a measure of CYP2B activity, were increased about five fold in the males at 250 ppm, however, the increase in the females (250 ppm) was only two fold. Relatively small changes were observed in chlorzoxazone hydroxylation (CYP2E1) in the male high dose group, while there were no differences in the females. Conversely, nifedipine oxidation (CYP3A4) showed a small increase in the females at 250 ppm and no change in the males. The relatively modest induction of a wide range of hepatic CYP-450 activities was limited to rats exposed to the 250 ppm dose of PCBTF. In addition, the results suggest that there are gender differences associated with the induction of CYP-450 activities.

1424 HEPATIC AND THYROID EFFECTS OF LY300164 SECONDARY TO HEPATIC MICROSOMAL ENZYME INDUCTION IN THE RAT

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Hepatic and thyroid changes were observed when the neuroprotectant LY300164 was given to Fischer 344 rats by oral gavage in daily doses of 10, 25 and 60 mg/kg for 31 days. Liver weights were increased by up to 45%, and were accompanied by cytomegaly, consistent with induction of microsomal drug-metabolizing enzymes. The hepatic content of cytochromes P450 was markedly increased, as were the enzymatic activities 7-ethoxyresorufin O-deethylase, benzphetamine N-demethylase, p-nitrophenol hydroxylase, and erythromycin N-demethylase. The pharmacokinetics of LY300164 were altered during the course of the study. The plasma AUC for LY300164 in the high dose group was reduced by about 4-fold after 14 days of dosing, when compared to a single dose, which indicates that the drug induced its own metabolic clearance. Thyroid weights were increased by up to 35%, and evidence of slight thyroid hyperplasia was observed in male rats. The possibility that the thyroid changes were caused by increased rates of thyroid hormone metabolism was supported by the finding of increased hepatic microsomal thyroxine-glucuronosyl transferase activity. These findings indicate that the induction of microsomal drug metabolizing enzymes by LY300164 led to increased hepatic clearance of the drug and of thyroxine; secondarily, increased rates of thyroxine clearance led to thyroid stimulation and hypertrophy.

1425 ASCORBIC ACID DEFICIENCY DECREASES THE EXPRESSION OF CYP4A1 IN LIVER MICROSOMES OF GUINEA PIGS

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The cytochrome P-450 monooxygenase system plays a central role in the oxidation of a wide variety of structurally unrelated compounds. The terminal oxidase, which represents a super family, exists in multiple forms. Its contribution is affected by nutritional and several other factors. We, and others, have shown that ascorbic acid (AA) deficiency decreases the content of cytochrome P-450 in liver microsomes of guinea pigs (GPs). Included in the group of cytochromes P-450 are the phenobarbital- and 3-methyl cholanthrene inducible moieties. In the present study the effect of AA status on another specific cytochrome P-450, CYP4A1, laurate ω-hydroxylase was investigated. Ascorbic acid may selectively increase or decrease certain forms of cytochromes. For four weeks adult male Hartley GPs were fed a diet containing 0[O-AA]; 0.1[L-AA] and 2.5%[H-AA]AA. The liver microsomes were isolated at this stage and cytochrome P-450 content was determined. O-AA group showed a significant decrease in cytochrome P-450 compared to L-AA and H-AA groups. They also showed a marked decrease in aminopyrine N-demethylase activity and cytochrome b_5 content. The expression of CYP4A1 was evaluated using Western blot and anti-CYP4A1 antibody. Group O-AA GPs showed a marked decrease in CYP4A1 expression. Groups L-AA and H-AA showed similar expression. This study demonstrated that CYP4A1, a specific cytochrome induced by hypolipidemic agents, is decreased by AA deficiency. The induction of CYP4A1 is known to cause hypolipidemia. Its markedly decreased expression in AA-deficient GPs may explain the occurrence of hyperlipidemia in these animals.

1426 EFFECT OF 3-METHYLCHOLANTHRENE ON TOXICITY OF SANGUINARINE IN MICE

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Sanguinarine is a major alkaloid found in the plant bloodroot (Sanguinaria canadensis L.). Extracts of the plant have been used in homeopathic medicine, toothpastes and oral rinse products. As regards its toxicity, it has been reported to cause numerous outbreaks of human poisoning known as epidemic dropsy. The present studies were conducted to examine the effect of sanguinarine on liver and its monooxygenases in mice. Administration of sanguinarine (10 mg/kg, ip) to male mice resulted in a significant decrease in monooxygenase activities and liver gluthathione content and an increase in the activities of SDH, ALT and AST in the serum. However, pretreatment of mice with 3-methylcholanthrene (3-MC) at 20 mg/kg/d, ip, for 3 days alleviated the aforementioned toxic effects of sanguinarine suggesting 3-MC may have induced a cytochrome P-450 species that detoxifies sanguinarine.

1427 DISTRIBUTION OF CYP 2E1 IN RAT TRACHEOBRONCHIAL AIRWAYS: RESPONSE TO CHRONIC OZONE EXPOSURE

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The distal trachea and centriacinus of the lung are primary sites injured during acute O_3 exposure; chronic exposure results in cells which are resistant to high doses of oxidant. The primary sites for ozone injury are also targets for a number of chemicals which undergo cytochrome P450 monooxygenase-dependent activation. These studies were designed to examine the effects of chronic O_3 exposure on pulmonary P450 in well defined subcompartments of the lung. Microsomes (20–50 μg protein) were prepared from these subcompartments. Metabolism of p-nitrophenol, a selective substrate for CYP 2E1, was assessed in these microsomes using HPLC with electrochemical detection of the nitrocatechol. The method is sensitive to 3 pmoles nitrocatechol. CYP 2E1 activities were highest in the distal bronchiole and minor daughter subcompartments, but were much lower in major daughter, lobar bronchus and trachea. This pattern of enzyme distribution was consistent with the distribution of protein immunochemically. Chronic exposure of rats to O_3 (90 days at 1 ppm) resulted in increased levels of expression of CYP 2E1 protein in the distal bronchiole in comparison to control. In contrast, the microsomal activities of CYP 2E1 were decreased by greater than 50% in the distal bronchiole in O_3 exposed rats in comparison to control. These studies indicate that CYP 2E1 activities vary substantially by airway level and that chronic ozone exposure results in an apparent increase in immunochemically detectable CYP 2E1 but a decrease in the activity of this isozyme. Supported by NIEHS 00628, ES 05707 and ES 03656.

1428 *IN VITRO* FORMATION OF A NOVEL THIOCARBAMIDE-GLUTATHIONE ADDUCT BY RAT LIVER MICROSOMES

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Microsomal metabolism of thiocarbamide compounds proceeds via sulfur oxidation to form reactive sulfenic and sulfinic acids. These reactive metabolites may covalently bind to intracellular proteins and lead to toxicity. A protective mechanism against this covalent binding has been proposed in which glutathione (GSH) causes reduction of the sulfenic acids to the parent thiocarbamides. This mechanism, however, may initiate a futile cycle whereby one molecule of thiocarbamide participates in the oxidation of many molecules of GSH. In the past study, *in vitro* evidence for an alternative mechanism is presented, which involves the formation of a novel thiocarbamide-glutathione adduct. Incubation of 100 μM [^{14}C] SDZ 266–376 with rat liver microsomes (2 nmol P450/mL, 1 mM NADPH, 30 min, 37 °C) resulted in covalent binding of radioactivity to microsomal protein as well as the formation of several products consistent with initial formation of sulfenic and/or sulfinic acids. In the presence of GSH (100 or 500 μM), however, covalent binding to protein was abolished and only one major NADPH-dependent product was formed. This product was subsequently identified as the glutathione adduct of SDZ 266–376 (I) by the following methods: (a) microsomal incubation of cold SDZ 266–376 in the presence of [^{35}S] GSH produced a product chromatographically identical to I, (b) chemical synthesis of I yielded a product with an identical MS/MS spectrum to that obtained from the microsomal incubations, (c) the MS/MS fragmentation pattern of the synthetic adduct was fully consistent with the structure and (d) interpretation of the NMR spectra of the synthetic adduct confirmed the assigned structure. We propose that GSH may participate in the consequences of oxidative thiocarbamide metabolism by adduct formation mechanisms.

1429 REDOX AND THE COVALENT BINDING OF ACETAMINOPHEN (APAP) TO MITOCHONDRIAL ALDEHYDE DEHYDROGENASE

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Covalent binding of APAP to mitochondrial proteins has been postulated to alter the function of the organelle and contribute to the hepatotoxicity upon APAP overdose. To help identify the arylated proteins, CD-1 mice were administered 600 mg/kg APAP for 4 hrs and Western blots of mitochondrial proteins were probed with anti-APAP Ab. Of the 5 arylated proteins (M.W of 75, 60, 54, 44 and 33 kDa) detected on 1-D gels, the 54 kDa target was most prominent. Upon submitochodrial purification using digitonin, sonication and differential centrifugation, this target was found to be most highly enriched in the inner membrane fraction. On 2-D gels 3 54 kDa arylated proteins with pIs of 6.4, 6.6 and 7.1 were detected. The pI 7.1 protein was excised and, after tryptic digestion, the 2 best resolved peptides were sequenced and found to be 100% homologous to mitochondrial aldehyde dehydrogenase. Although the activity of this enzyme is decreased by 45% at 4hrs after APAP administration, the mitochondrial redox (measured by NAD/NADH ratio) was actually decreased. This data may shed light on the impaired mitochondrial function associated with APAP induced hepatotoxicity. (NIH GM-31460)

1430 COVALENT BINDING OF DICLOFENAC TO CANALICULAR LIVER PLASMA MEMBRANE PROTEINS IN WISTAR BUT NOT IN TRANSPORT-DEFICIENT (TR$^-$) RATS

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The NSAID diclofenac is bioactivated to its acyl glucuronide which covalently binds to target proteins in rat liver cell plasma membranes (LPM). To further investigate the role of the canalicular (c) multispecific organic anion transporter (MOAT) in covalent binding of diclofenac, we compared protein binding of diclofenac in wild-type (Wistar) with that in mutant transport-deficient (TR$^-$) rats. Cultured hepatocytes isolated from both strains were exposed to nontoxic concentrations (100 μM) of [^{14}C]-diclofenac. Total binding to protein was reduced by 75% in hepatocytes from TR$^-$ versus Wistar rats. Furthermore, dibromosulphophthalein, a non-metabolizable substrate of the MOAT, inhibited in a concentration-dependent manner the covalent binding of diclofenac to hepatocellular protein. Finally, isolation of cLPM and basolateral (bl) LPM from the livers of rats treated with diclofenac (30 mg/kg/d x 3d) revealed selective protein adduct formation (apparent M_r 126 kD) to cLPM, but not blLPM, from Wistar rats. In contrast, binding in TR- rats was not detectable in Western blots probed with a specific anti–diclofenac antibody. Collectively, these results demonstrate that active canalicular transport is necessary for covalent protein adduct formation of diclofenac glucuronide.

1431 USE OF PROTEIN ADDUCTS TO INVESTIGATE THE REACTIVE METABOLITES OF BENZENE IN RATS

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Adducts of hemoglobin (Hb) and albumin, arising from reactions with several electrophilic metabolites of benzene (i.e., benzene oxide, 1,2-benzoquinone (1,2-BQ), and 1,4-benzoquinone (1,4-BQ)) were used to study the disposition of reactive metabolites following administration of benzene to F344 rats (single oral dosage of [$^{13}C_6$]benzene and/or [^{14}C]benzene at 0, 50, 100, 200, or 400 mg/kg body weight). The blood proteins were isolated and analyzed for both total protein binding (as determined by scintillation counting) and cysteine-bound adducts. Adducts of 1,2-BQ and 1,4-BQ were determined by GC-MS following catalytic cleavage with Raney nickel followed by derivatization with heptafluorobutyrylimidazole while benzene oxide adducts were simultaneously cleaved off the protein and derivatized with trifluoroacetic anhydride. Although albumin and Hb contained similar levels of total

bound metabolites, the levels of the specific adducts varied between the proteins. Thus, the use of both albumin and Hb adducts was found to provide more information about the disposition of the various benzene metabolites in vivo than would be obtained by investigating only a single protein. This research is supported by NIEHS grant P42ESO5948.

1432 ROLE OF HYPEROXIA IN THE OXYGEN-ACTIVATION OF SUPEROXIDE DISMUTASES IN THE SYMBIOTIC DINOFLAGELLATE SYMBIODINIUM MICROADRIATICUM

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Symbiotic dinoflagellates (*Symbiodinium* spp.) live in coral reef habitats characterized by daily exposure to hyperoxia and hypoxia (0–78% pO_2). In an earlier study, (J. Phycol. 28:343–346, 1992) it was shown that *Symbiodinium microadriaticum* contains three superoxide dismutase (SOD) isoenzymes; CuZnSOD, MnSOD, FeSOD. When this species was cultured under hyperoxic (63% pO_2) conditions, an increase of 35% in SOD specific activity (water-soluble fraction) was found relative to normoxic (21% pO_2) controls. Under normoxic conditions, MnSOD contributes approximately 50% of the total SOD specific activity whereas the remaining activity is due to CuZnSOD (25%) and FeSOD (25%). MnSOD is the most responsive SOD isoenzyme to hyperoxia in *S. microadriaticum*. Experiments were undertaken to study how MnSOD activity is regulated in cells exposed to constant hyperoxia (10–14 days). First, MnSOD protein was purified from *S. microadriaticum* to electrophoretic homogeneity by means of ammonium sulfate fractionation, isoelectric focusing electrophoresis and column chromatography. Polyclonal antibodies against the MnSOD antigen were subsequently raised and purified by immunoaffinity chromatography. Dose-response curves were established for hyperoxia (42%, 63%, 84%, 99.5% pO_2) and changes in levels of MnSOD (measured by specific activity and by immunoblots). Collectively, these studies support the hypothesis that *S. microadriaticum* contains a sensitive mechanism of regulating MnSOD activity that is at least partially responsible for its survival in hyperoxic habitats. Supported by grant NIH-MBRS 5 S06 GM08239- 10 and NIH-RCMI SG12RR03050–10.

1433 IMMUNOCHEMICAL VISUALIZATION OF RAT LIVER PROTEINS ADDUCTED BY BUTYLATED HYDROXYTOLUENE (BHT)

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Several alkylphenols, such as butylated hydroxytoluene (BHT), form reactive quinone methide intermediates upon oxidation by cellular enzymes. In order to pursue the role of protein alkylation in alkylphenol toxicity we used an immunochemical approach to identify protein targets alkylated by BHT. Synthetic BHT/N-acetylcysteine was coupled to keyhole limpet hemocyanin and used as an antigen from which polyclonal antibodies were raised in NZW rabbits. Rabbit sera contained an antibody which was highly specific for BHT, as determined by competitve ELISA. The BHT antibody was used as a probe to look for the presence of BHT-protein adducts in *in vitro* incubations with rat liver microsomes or tissue slices and also *in vivo* in liver tissue from male Sprague-Dawley rats exposed to BHT. Western blotting of protein gels revealed BHT-dependent protein binding over a wide molecular weight range. Although binding was somewhat dependent on the experimental system used, prominent recurrent bands were observed at approximately 97, 74, 64, 52, 50 and 34.5 kD. Detection of adducts was inhibited in microsomal incubations by cytochrome P450 inhibitors, omission of NADPH, and deuterated BHT. Studies are currently in progress to determine the identity of these target proteins. (Supported by ES06016).

1434 METHYL PALMITATE (MP) DIMINISHES SELECTIVE PROTEIN ARYLATION AND PROTECTS AGAINST ACETAMINOPHEN (APAP) HEPATOTOXICITY IN MALE CD-1 MICE

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In rats, activation of hepatic macrophages has been implicated in APAP hepatotoxicity. To investigate this in an established mouse model of APAP hepatotoxicity, MP was used to inhibit macrophage function. Mice were dosed *iv* with 57mg MP or 0.7 ml 5% dextrose, 0.1% Tween-20 vehicle. MP altered macrophage function as measured by a > 40% decrease in carbon clearance in unchallenged mice. Other mice were challenged 24 hr later, after an overnight fast, with APAP(600mg/kg) or 50% propylene glycol/ water vehicle *ip*. Covalent binding and toxicity were later assessed at 4 and 12 hrs respectively. MP pretreatment decreased APAP-induced toxicity as indicated by histopathology and diminished sorbitol dehydrogenase leakage (from 2530 ± 377 to 668 ± 17 U/L). MP pretreatment did not alter APAP-induced non-protein sulfhydryl depletion. However quantitative immunochemical analysis with the anti-APAP and anti-58-ABP anti-sera revealed that the relative extent of APAP binding ($^{APAP}/_{58\text{-}ABP}$) to the major protein target, the 58-ABP, was significantly lower in the MP/APAP group. Thus factors other than altered macrophage function (e.g. modified specific protein arylation), may also contribute to the protective effect of MP in APAP-induced hepatotoxicity. [NIGMS31460, NIEHSO7163, UCONN Center for Biochem. Tox.; Fulbright-LASPAU Scholarship (WSB)]

1435 FREE RADICAL GENERATION IN HAMSTER LUNG AND LIVER MICROSOMES INCUBATED WITH AMIODARONE

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Amiodarone is an efficacious antidysrhythmic agent. However, associated with its use, is its ability to produce numerous adverse effects including life threatening hepatotoxicity and pulmonary fibrosis. Free radical production has been proposed as a possible mechanism involved in initiating toxicity. Utilizing electron spin resonance spectroscopy and the spin trapping agent, C-phenyl-N-tert-butyl nitrone (PBN), a radical-PBN spin adduct was measured following 1-hr incubation of hamster liver or lung microsomes with amiodarone in the presence of NADPH. The hyperfine splitting constants determined for this species ($a^N = 14.2$ and $a^H_\beta = 2.2$) were consistent with the formation of a carbon-centred radical. The magnitude of radical formation was dependent on both amiodarone and NADPH concentrations. A radical-PBN spin adduct was also measured following incubation of liver microsomes with desethylamiodarone, a metabolite of amiodarone. These results suggest that a carbon-centred free radical mechanism may be involved in amiodarone-induced pulmonary and hepatic toxicities. (Supported by Medical Research Council of Canada Grant No. MT-13257)

1436 GLUTATHIONE-MEDIATED FORMATION OF A CYCLIC 1,N²-PROPANODEOXYGUANOSINE ADDUCT FROM 2-BROMOACROLEIN

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The mutagen 2-bromoacrolein (2-BA) is a major P450 oxidative metabolite of 1,2-dibromo-3-chloropropane (DBCP) and tris (2,3-dibromopropyl)phosphate (Tris-BP). We have recently reported that 2-BA reacted *in vitro* with glutathione (GSH) to yield an α, β-unsaturated aldehyde and other reactive GSH conjugates that may covalently bind to deoxyguanosine (dG). The present study was conducted to characterize the structure of a GSH-mediated 2-BA adduct to dG and the molecular mechanism of its formation. Electrospray LC/MS was employed to analyze GSH conjugates and dG adducts formed from incubations of 2-BA with GSH and dG at pH 7.4. Subsequent to the formation of GSH conjugates of 2-BA, a major dG adduct was observed. Based upon characterization by UV and ^{1}H-NMR spectroscopy and by tandem mass spectrometry, this adduct was identified as 6-*S*-glutathionyl-8-hydroxy-1, N^2-propanodeoxyguanosine. The structure of the cyclic dG adduct suggests that *S*-(3-oxopropenyl)glutathione, a reactive GSH conjugate of 2-BA, is the intermediate that reacts with dG. Direct evidence for *S*-(3-oxopropenyl)glutathione-mediated formation of the dG adduct was established by the observation that the same cyclic dG adduct was formed when the synthesized GSH conjugate was allowed to react with dG. These results suggest a novel GSH-mediated bioactivation pathway for DBCP and Tris-BP that may play a role in DBCP and Tris-BP-mediated DNA damage. (This work was supported by NIH Grant No ES-02728)

1437 NEPHROTOXICITY WITH ABSENCE OF HEPATOTOXICITY IN CD-1 MICE GIVEN ACETAMINOPHEN (APAP) AND N-ACETYLCYSTEINE (NAC)

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N-acetylcysteine (NAC) is the recommended antidote for overdose with APAP and prevents its toxicity in man and experimental animals. In some cases of human APAP overdose NAC prevented toxicity in liver but not in kidney. These studies were conducted to compare the effectiveness of NAC as an antidote for APAP induced hepatic and renal toxicity in male CD-1 mice. Selected doses of NAC or water (DDW) vehicle (oral, 10 ml/kg) were given prior to challenge with 600 mg APAP/kg or 50% propylene glycol (PG) vehicle (ip, 10 ml/kg). Mice were killed 4 or 12 hrs later for evaluation of protein arylation and toxicity, respectively. A single dose of 600 mg NAC/kg 5 min prior to APAP challenge, completely prevented protein arylation and toxicity in the liver but had no apparent effect in the kidney (sorbitol dehydrogenase (SDH) $\bar{x}$ = 2216 vs 2 U/l and plasma urea nitrogen (BUN) $\bar{x}$ = 44 vs 48 mg/dl for APAP and NAC/APAP treated mice, respectively). Histopathological evaluation of the tissues confirmed that NAC protected from hepatotoxicity but not nephrotoxicity. Similarly, a 5 min pretreatment with 1 g NAC/kg did not prevent APAP induced kidney toxicity (BUN $\bar{x}$ = 38 and 32 mg/dl for APAP and NAC/APAP treated animals, respectively). Repeat dosing with NAC (600 mg/kg) 5 min prior and 30 min, 1 hr and 2 hr after APAP also prevented hepatotoxicity but had no apparent effect on APAP-induced protein arylation or toxicity in the kidney (BUN $\bar{x}$ = 57 and 53 mg/dl for APAP and NAC/APAP treated animals, respectively). These findings demonstrate that APAP toxicity with the CD-1 mouse models the reports of NAC protection of hepatotoxicity but not nephrotoxicity in some cases of human APAP overdose. [NIGMS31460, NIEHS07163, UCONN Center for Biochem. Tox. and Research Foundation, Boehringer Ingelheim Predoctoral Fellowship (DJH)]

1438 ENHANCEMENT OF PEROXIDASE-CATALYZED XENOBIOTIC OXIDATION BY PHENOTHIAZINES

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Many drugs, pollutants, and other xenobiotics are known to be oxidized by peroxidases to potentially harmful free radical intermediates. We explored the hypothesis that certain compounds, acting as efficient peroxidase substrates, may stimulate the formation of other reactive free radicals by acting as mediators of electron transfer reactions. We investigated the interaction of two well-known peroxidase substrates, chlorpromazine (CPZ) and aminopyrine (AP). As shown by ESR and UV-vis spectroscopy, chemically generated chlorpromazine radical (CPZ.+) was able to oxidize AP to its cation radical (AP.+). The rate constant for this reaction was estimated to be 1 X 107 M-1 S-1 at pH 4.5. Kinetic studies showed that rate constants for CPZ oxidation to CPZ.+ by horseradish peroxidase (HRP) were about 100-fold greater than the corresponding rate constants for oxidation of AP to AP.+. When both CPZ and AP were present with HRP and H_2O_2, AP.+ formation was stimulated 100-fold. Concomitantly, the accumulation of CPZ.+ was completely inhibited by AP. Other phenothiazines behave similarly to CPZ in stimulating HRP-catalyzed oxidation of AP. Similar results were also obtained using lactoperoxidase, and the myeloperoxidase mimic, HOCl, in place of HRP. These data suggest that phenothiazines can act as redox mediators to stimulate peroxidase-catalyzed oxidation of AP and other chemicals to potentially toxic free radical intermediates. This mechanism also suggests that chemicals like AP may interfere in the metabolism of phenothiazines by peroxidases. Given the nature of the enzymes and chemicals involved, this mechanism may have implications for drug-drug interactions, idiosyncratic drug reactions and carcinogenesis.

1439 LOW SUSCEPTIBILITY TO 3-METHYLINDOLE-MEDIATED TOXICITY CORRELATES TO HIGH DETOXICATION BY ALDEHYDE OXIDASE

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The objective of this study was to determine if aldehyde oxidase (AO) is involved in the detoxication pathway of a reactive 3-methylindole (3MI) metabolite. 3MI is a selective pulmonary toxin; no observable hepatic toxicities have been demonstrated in any species. Species susceptibility to 3MI-induced pneumotoxicity is goat>rat/mouse>rabbit. At least 2 reactive intermediates are formed during 3MI metabolism. One of these intermediates is a cytochrome P450 oxidation product of 3MI via rearrangement of the 2, 3 epoxide, 3-hydroxy-3-methylindolenine (HMI). HMI is likely to be partially responsible for the pulmonary toxicity of 3MI due to the binding of this electrophile to proteins or glutathione. AO could be an important modulator of this toxicity because the enzyme would detoxify the HMI intermediate. Incubations of cytosolic aldehyde oxidase from lung and liver of goat, rat, mouse and rabbit demonstrated that the relative rates of HMI detoxication to 3-hydroxy-3-methyloxindole paralleled the order of species and organ susceptibility to 3MI. Therefore, deficient detoxication of HMI appears to be a major factor in the higher susceptibility of ruminant and rodent lungs to 3MI-induced damage. (Supported by USPHS grant HL13645 from the National Heart, Lung, Blood Institute).

1440 COVALENT BINDING OF A QUINONE METHIDE TO MOUSE LUNG PROTEINS

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Previous studies with rat hepatocytes, mouse keratinocytes, and mouse bronchiolar Clara cells strongly implicate quinone methides as toxic metabolites of phenolic compounds. These reactive species are generated through cytochrome P450 mediated electron transfer oxidation, and readily form covalent adducts with thiols and, to a lesser extent, with amino groups. In mouse lung, 2,6-di-*tert*-butyl-4-methylphenol (BHT) promotes carcinogen-induced tumors, and our evidence indicates that hydroxylation of a *tert*-butyl group by P450 to form BHTOH is a necessary first step in BHT promotion. The second step appears to involve P450-dependent oxidation of BHTOH to its quinone methide (QMOH). ^{14}C-labeled BHT or BHTOH was incubated with 9000g supernatant fractions of DBA mouse lung and with both compounds, QMOH formation was verified by HPLC analyses of the resulting GSH conjugate. Cytosolic proteins were separated and covalently-bound radioactivity measured. Binding was greatly inhibited by heat-inactivation or with the P450 inhibitor SKF 525A. Protein analysis by SDS PAGE demonstrated binding to five proteins corresponding to the following approximate molecular weights (% of total ^{14}C): 22 kD (17.6), 45 kD (52.5), 55 kD (16.0), 64 kD (8.6), and 84 kD (5.3). The relative amounts of radioactivity associated with these bands roughly correlated with the amount of protein, except for the 45 kD protein (pI = 4.80) which was labeled to a much higher degree than the others. Preliminary evidence indicates that this may be a protein involved in signal transduction, thereby suggesting a possible link to BHT tumor promotion. (Supported by NIH grant ES06216)

1441 TISSUE - SPECIFIC 4-AMINOBIPHENYL DNA ADDUCT FORMATION IN THE Cypla2(-/-) KNOCKOUT MOUSE

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The metabolism of the human urinary bladder carcinogen, 4-aminobiphenyl (4-ABP), has been extensively studied in humans and rodents. CYP1A2 is the primary cytochrome P450 which N-oxidizes 4-ABP to form DNA adducts. However, CYP1A1, CYP2B1 and CYP2B2 also N-oxidizes 4-ABP. Using a *Cypla2(-/-)* knockout mouse, we have examined the role that these cytochrome P450's play in 4-ABP DNA adduct formation. *Cypla2*(+/+) mice and *Cypla2*(-/-) mice were dermally treated with 200 nmoles of 4-ABP daily for 5 days. DNA and microsomes were isolated from liver, skin, urinary bladder and lung. DNA adduct levels in each of the tissues were determined by ^{32}P postlabeling. CYP1A1, CYP1A2, CYP2B9 and CYP2B10 (mouse orthologues to rat CYP2B1 and CYP2B2) expression was determined in each tissue by western blotting. 4-ABP DNA adduct levels were higher in the *Cypla2*(+/+) urinary bladder and skin, compared with the levels in the *Cypla2(-/-)* tissues. However 4-ABP DNA adduct levels in the liver were aproximately equal. The results indicate that, while CYP1A2 is the main activating P450 in 4-ABP metabolism, CYP1A1, CYP2B1 and CYP2B2 may produce reactive 4-ABP metabolites as well. Supported by NIH grants F30 ES06096, R01 ES06321 and T32 ES07278.

1442 EVIDENCE FOR THE PRESENCE OF NICOTINE IMINIUM IONS IN AREAS OF RAT BRAIN USING THE CYANIDE TRAPPING TECHNIQUE AFTER INTRAPERITONEAL ADMINISTRATION OF NICOTINE

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Nicotine is metabolised to cotinine via the formation of nicotine-1'5'-iminium ion. This reactive intermediate can be trapped with cyanide ions as the corresponding 5'-cyanonicotine. Similarly 1'-methylene and 2'-iminium ions of nicotine can be detected as the corresponding cyano derivatives. In an effort to examine the formation and role of iminium ions in the metabolism of nicotine, 2'-cyanonicotine, 1'-cyanomethyl-nornicotine and the diastereoisomers of 5'-cyanonicotine were prepared for use as analytical standards. Male Wistar rats (250-290g) were injected with nicotine (i.p. 9.75mg/Kg) and killed by cervical dislocation after 20 minutes. The brain was removed and dissected into six parts (cortex, cerebellum, striatum, hippocampus, hypothalamus and rest of the brain). The livers, kidneys and lungs were also removed. The tissue from 10 rats was bulked together and homogenised with potassium cyanide solution (0.8mM) and the homogenates stored at -20°C prior to analysis. Analysis of chloroform extracts using HPLC (UV detection) and GC (NPD detection) revealed the presence of 5'-cyanonicotine and 1'-cyanomethylnornicotine in liver, kidney and lung homogenates. In the case of brain homogenates: none of the cyano derivatives of nicotine iminium ions were observed in the hippocampus or the striatum; 1'-cyanomethylnornicotine only was detected in the cerebellum; 5'-cyanonicotine and 1'-cyanomethylnornicotine were observed in very small amounts in the cortex and hypothalamus, whilst higher amounts were detected in the rest of the brain. At present it can not be concluded whether nicotine iminium ions pass through the blood-brain barrier or are produced by brain metabolism. Studies, are currently being carried out to resolve this and determine whether specific regions of the brain are responsible for the generation of nicotine iminium ions.

1443 HEMOGLOBIN ADDUCTS FROM ACRYLONITRILE AND ETHYLENE OXIDE IN CIGARETTE SMOKERS: EFFECTS OF GLUTATHIONE TRANSFERASE T1 AND M1 GENOTYPES

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Acrylonitrile (ACN), used to manufacture plastics and fibers, is carcinogenic in rats and is found in cigarette smoke. Ethylene oxide (EO) is carcinogenic in rodents, and is a metabolite of ethylene, also found in cigarette smoke. Both ACN and EO undergo conjugation with glutathione. To determine whether glutathione transferase (GST) genotype for GST M1 or T1 alters the internal dose of these agents, hemoglobin adducts from EO and ACN were measured in smokers and non-smokers. *N*-(2-cyanoethyl)valine (CEVal) formed from ACN and *N*-(2-hydroxyethyl) valine (HEVal) from EO were measured in globin from 16 non-smokers and 33 smokers (1–2 pack/day). Questionnaire information on smoking habits was obtained. Plasma cotinine and GST genotypes for the T1 and M1 forms were determined. Both CEVal and HEVal levels increased with smoking and with cotinine. CEVal and HEVal were also correlated. GST M1 and T1 genotypes had little effect on CEVal. GST M1 had little effect on HEVal. However, HEVal was elevated in GST T1-individuals when normalized to smoking status or cotinine levels (HEVal: cotinine in T1- smokers, 1.58 ±0.47; in T1+ smokers, 1.08 ± 0.54; p= 0.03). The ratio of HEVal:CEVal was also elevated in GST T1-smokers (1.50 ± 0.56 vs. 0.88 ± 0.24; p = 0.0005). The presence of GST T1 appears to reduce the internal dose of EO by approximately one-third.

1444 IMMUNOCHEMICAL DETECTION OF 4-HYDROXYNONENAL AND MALONDIALDEHYDE HEPATIC PROTEIN ADDUCTS IN LIVER SECTIONS AND SUBCELLULAR FRACTIONS ISOLATED FROM RATS TREATED WITH CARBON TETRACHLORIDE

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The ability of agents such as carbon tetrachloride (CCl_4) to induce hepatic lipid peroxidation (LPO) is well described. Metabolism of CCl_4 initiates the oxidative breakdown of polyunsaturated fatty acids producing α,β-unsaturated aldehydes, such as 4-hydroxynonenal (HNE) and malondialdehyde (MDA). The facile reactivity of these electrophilic aldehydes may play a role in the toxicity of compounds like CCl_4. To examine the time course at which CCl_4 associated HNE and MDA alkylate hepatic protein, male HAS rats were given an acute intragastric dose of CCl_4 (0.1 ml/kg) and sacrificed 0–72 hrs after administration. Rabbit polyclonal antisera directed toward HNE-thiol epitopes or MDA-amine epitopes were employed in immunohistochemical and immunoprecipitation analyses to detect HNE and MDA protein adducts in paraffin embedded liver sections and isolated subcellular fractions. As early as 2.5 hrs post CCl_4, HNE and MDA adducts were detected immunohistochemically in hepatocytes localized to zone 2. Further exposure to CCl_4 caused an increase in immunopositive hepatocytes over 24 hrs in zone 2 as well as zone 3. Immunopositive cells were cleared from the lobule by 36 to 72 hours. Anti-MDA-amine serum immunoprecipitated a number of proteins in liver fractions at various time points after CCl_4 administration. These include four proteins in liver homogenate (~132–180 kDa; 18–36 hrs), a microsomal protein (~100 kDa; 6 hrs), and a mitochondrial protein (~75 kDa; 24–36 hrs). Anti-HNE-thiol serum immunoprecipitated a protein in liver homogenate (~150 kDa; 36 hrs). These results demonstrate that HNE and MDA alkylate hepatic tissue which may be associated with the degeneration of the liver during CCl_4 exposure. (NIH/AA05370 and AA9300)

1445 STUDIES ON THE MECHANISM OF HEPATOTOXICITY OF p-CRESOL: EFFECTS OF DEUTERIUM LABELLING AND RING SUBSTITUTION

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We recently observed that *p*-cresol (4-methylphenol) is the most toxic cresol isomer in rat liver tissue slices. A possible mechanism involves biotransformation of *p*-cresol to a reactive quinone methide intermediate which covalently binds to cellular macromolecules and elicits cell death. In order to obtain further evidence for this proposed mechanism, we studied the effects of deuteration of the methyl group of *p*-cresol, and the presence of various ring substituents, on the metabolism and toxicity of *p*-cresol in precision-cut male Sprague-Dawley rat liver slices. Deuterium-labelled *p*-cresol was significantly less toxic than the parent compound in rat liver slices after 6 hr incubation (LC_{50}=3.36 mM and 1.31 mM, respectively). In addition, deuterated cresol was metabolized to a reactive intermediate at a rate approximately 50% of that of *p*-cresol in both liver slices and liver microsomal incubations (measured as glutathione conjugate formation). The presence of electron withdrawing substituents (2-chloro or 2-bromo) markedly enhanced both metabolism and toxicity, with the exception of 2,6-dibromocresol, which was similar to cresol in terms of rate of metabolism and toxicity. Conversely, the presence of electron donating substituents (2-methoxy, 2-methyl or 2,6-dimethyl) diminished metabolism and toxicity. Methylation of the hydroxyl group to form 4-methylanisole, also reduced toxicity. These results support the hypothesis that the toxicity of *p*-cresol is dependent on the formation of a reactive quinone methide intermediate (ESO6016).

1446 ACETAMINOPHEN (AP) AT THERAPEUTIC DOSES (FOR HUMANS) COVALENTLY BINDS TO DNA IN MICE

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Administration of large doses of AP to experimental animals results in apparent alkylation of DNA. In the present study we investigated the dose-response of binding to DNA in male ICR mice. Mice were treated with 10, 20, 50, or 100 mg/kg AP (with 1-2 mCi/mouse of ^{3}H-AP), sacrificed at 2 h, and hepatic DNA isolated by organic extraction. We added appropriate amounts of ^{3}H-AP to proteinase-treated liver homogenates of untreated mice, isolated DNA and found no measurable radioactivity above background. In contrast, all animals treated *in vivo* showed ^{3}H associated with isolated DNA (4 to 20 times 20 cpm background). Levels of binding, in pmol/mg of DNA were (mean ± SEM, n = 3–7): 2.0 ± 0.4; 1.6 ± 0.6; 6.6 ± 2.3; and 8.1 ± 2.7, respectively. This apparent alkylation of DNA by AP reactive metabolites is observed at doses that are commonly ingested by humans and are far below those required to exceed the threshold to GSH depletion, protein alkylation, and acute cell death in this animal model. In addition to the question of initiation of neoplasia, the present data are also interesting in view of a recent epidemiological study showing a strong association between end-stage renal disease in humans and cumulative dose of AP both in daily consumption and total lifetime exposure [NEJM 331: 1675]. Supported by NIH GM44263.

1447 TARGETS OF A 3-METHYLINDOLE REACTIVE INTERMEDIATE ARE CHARACTERIZED BY A SELECTIVE ANTIBODY

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3-Methylindole (3MI) selectively causes damage to pulmonary tissues. 3MI is bioactivated to toxic intermediates by cytochrome P450 enzymes. Covalent binding of the electrophilic 3-methyleneindolenine intermediate to proteins is a plausible mechanism of 3MI-mediated lung damage. An antibody was developed to thio-ether adducts of 3-methyleneindolenine and shown by competitive ELISA to be highly selective for the detection of adducts of 3MI. Rabbits, rats and goats were treated with 350, 400, and 15 mg/kg (respectively) 3MI for 24 hours. The lung, liver, and kidney of each animal was collected and tissue fractions were analyzed by ELISA and western blot. Lung tissue from goat (pellet, cytosol, and microsomes) had greater immunoreactivity than rat. Rat was usually greater than rabbit. Normally in all the animals lung had greater immunoreactivity than kidney. Kidney had greater reactivity than liver. These studies demonstrate that immunoreactive 3MI adducts are produced in greater amounts in susceptible organs and species than in nonsusceptible cases. In addition a cytosolic 58 kDa protein is a major target of the electrophilic methyleneimine produced from 3MI. (Supported by USPHS grant HL13645 from the National Heart, Lung, and Blood Institute)

1448 BIOACTIVATION OF ESTRONE AND ITS CATECHOL METABOLITES TO QUINOID-GLUTATHIONE CONJUGATES IN RAT LIVER MICROSOMES

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There is a clear association between excessive exposure to synthetic and endogenous estrogens and the development of cancer in several tissues including breast, endometrium, liver and kidney. Although the carcinogenic effects of estrogens have been mainly attributed to hormonal properties, there is interest in estrogens acting as chemical carcinogens by binding to cellular macromolecules. In the present study, we explored factors which influence the rate of P450-catalyzed formation of the *o*-quinones from 2-hydroxyestrone (2-OHE) and 4-hydroxyestrone (4-OHE) as well as from estrone in rat liver microsomes. The initially formed *o*-quinones were trapped as their GSH conjugates which were separated and characterized by HPLC with electrospray-MS detection. Both 2-OHE and 4-OHE were excellent substrates for P450 generating *o*-quinone GSH adducts at 40 and 94 times respectively the rate of estrone. 2-OHE but not 4-OHE saturated P450 at unusually low concentrations (0.2 nmol P450/mL) perhaps due to differences in the stability of the *o*-quinones formed in the active site of the enzyme. The *o*-quinone from 2-OHE has a half-life of 42 $\pm$ 3 sec at 37 °C, pH 7.4 and the *o*-quinone from 4-OHE has a half-life of 12.2 $\pm$ 0.4 min under identical conditions. Finally, we showed that the *o*-quinones of two AB ring analogs of the catechol estrogens (3,4-dihydroxy-5,6,7,8-tetrahydronaphthylene and 1,2-dihydroxy-5,6,7,8-tetrahydronaphthylene) isomerize to quinone methides which implies that a similar reaction pathway could occur with the *o*-quinones from catechol estrogens.

1449 ACRYLONITRILE BIOACTIVATION; ROLE OF IRON/ HYPOXANTHINE/ XANTHINE OXIDASE SYSTEM IN VITRO

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Acrylonitrile (VCN) requires cytochrome P450 mediated oxidation to reactive intermediates in order to exert its genotoxicity. The aim of this study is to determine if other enzyme systems, known to produce reactive oxygen species (ROS), are capable of VCN oxidation. In this study iron/ hypoxanthine/ xanthine oxidase system was used, ROS formation (.OH) was monitored and VCN oxidation was determined by the rate of CN. released. In the incubation mixtures the rate of formation of .OH and that CN. was dependent on xanthine oxidase (XO) concentrations. Addition of sulfhydryl compounds (0.1mM); glutathione (GSH) or N-acetyl-L-cysteine (NAC) increased .OH formation (about 177% of control) and the rate of CN. release (about 200% of control). When various concentration of free radical scavengers; mannitol or dimethyl sulfoxide were added to the reaction mixtures .OH formation and the rate of CN. release were significantly reduced. Addition of antioxidant enzymes catalase (1000 U/ml) or superoxide dismutase (180 U/ml) significantly decreased the .OH formation (73% and 68% of control respectively) and reduced CN. release (11% and 60% of control respectively). Deferroxiamine (iron chelator) significantly decrease the .OH formation (85% of control) and CN. release (65% of control). Allopurinol, specific inhibitor of XO, significantly suppresed the .OH formation and the oxidation of VCN to CN.. Studies on the effect of various substrates of XO on the rate of VCN oxidation indicated that hypoxanthine is the most effective substrate. We conclude that free radicals generates by Fe/ HX/ XO system are directly implicated in the oxidation of VCN and that compansation of these radicals decrease the rate of CN. release. The oxidation of VCN by Fe/ HX/ XO system to potentially reactive intermediates represent a novel metabolic pathway for VCN bioactivation and may be important in the processes of VCN-induced carcinogenesis. [Supported by NIEHS Grant No. ES 01817].

1450 INHIBITION OF GLUTATHIONE (GSH) CONJUGATION *IN VIVO* AND IN THE PERFUSED RAT LIVER: STRUCTURE-ACTIVITY RELATIONSHIP FOR A SERIES OF GSH ANALOGUE ESTERS

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To assess the role of Glutathione S-transferases (GST) in the (de)toxification of substrates, selective GST inhibitors are required. We have designed an inhibitor based on the structure of GSH. The Cys-Gly moiety was replaced by D-α-aminoadipic acid (D-Aad). On the α-carboxyl group of D-Aad (corresponding to the -SH of cysteine), a branched 2-heptyl chain (R1) was introduced for interaction with the hydrophobic substrate binding domain on GST. The o-carboxyl of D-Aad (corresponding to the Gly carboxyl group of GSH) was esterified with an ethyl group (R2). This compound inhibits GSH conjugation in the rat in vivo (Biochem. J. 308, 283–290, 1995). In an attempt to increase the effectivity of this compound, the 2-heptyl moiety at R1 and the ethyl ester at R2 were replaced by longer alkyl chains. In addition, unesterified and diesterified analogues (ethyl esters at the α-carboxyl of Glu and at R2) were synthesized.

The inhibitory potency of the compounds towards the GSH conjugation of (*RS*)-2-bromoisovalerylurea (BIU) was evaluated in rat liver perfusions and in vivo. All compounds were selective inhibitors of (*S*)-BIU, which is a substrate of Alpha class GST. (*R*)-BIU, a substrate of Mu-class GST was not inhibited. All the mono ester compounds were equally effective, irrespective of the length of the alkyl chain. The un- and diesterified compounds were less potent than the mono ethyl ester. Compounds with variations at R1 were approximately equipotent.

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1451 SODIUM CYANATE REDUCES RAT BRAIN GLUTATHIONE REDUCTASE ACTIVITY

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Glutathione (GSH) plays an important role in the detoxification of xenobiotics, sequestration of free radicals and mitochondrial (mt) functions in brain. We reported earlier that sodium cyanate (NaOCN), an anti-sickling drug in the treatment of sickle-cell anemia, causes neurotoxicity and depletes GSH levels in mouse brain (Tor-Agbidye *et al.*, Soc. Neurosci. 1995). However, the mechanisms by which NaOCN reduces GSH levels and causes mt damage are unknown. This study investigates the possibility that NaOCN reduces GSH levels by inhibiting glutathione reductase (GR) or activating glutathione peroxidase (GP), two key enzymes in the regulation of intracellular GSH. Rats treated for 30 min with different doses of cyanide-free NaOCN (100 or 300 mg/kg, i.p.) showed ataxia, dysmetria, generalized weakness, and hyperreflexia. While brain GP was not inhibited, GR was reduced in striatum and hippocampus (~25% & ~60%, respectively) more than in cerebral cortex and cerebellum (~20% & ~40%). Neither GR nor GP was inhibited when brain post-mt fractions were incubated with 1–5 mM NaOCN. These data suggest NaOCN induces GSH deficiency by indirectly reducing GR, a state associated with neurologic dysfunction. [Supported by grant NS 19611 and a grant from E.I. Du Pont de Nemours].

1452 CLONING AND SEQUENCING OF ALPHA-CLASS GST CDNAS FROM THE MACACA LIVER

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The glutathione S-transferases (GSTs) are a multigene family of drug-metabolizing enzymes that catalyze the conjugation of reduced glutathione (GSH) with the electrophilic centers of a variety of xenobiotics. Thus, the tissue-specific expression of specific GST isoforms is a determinant of susceptibility to certain environmental chemicals, including the potent carcinogen aflatoxin B_1 (AFB_1). Mice are resistant to AFB_1-induced hepatocarcinogenesis due to the expression of an alpha-class GST isoform (mYc) that has high activity toward the reactive intermediate aflatoxin B_1-8,9-epoxide (AFBO). In contrast, rats do not constitutively express a GST isoform with high AFBO activity and are sensitive to AFB_1-induced hepatocarcinogenesis. However, neither rodents are appropriate models to study the hepatocarcinogenic effects of AFB_1 in humans. Because both humans and *Macaca fascicularis* exhibit similar hepatic GST activities toward AFBO, our laboratory is currently evaluating the suitability of this non-human primate as a model to study the AFB_1-induced hepatocarcinogenesis in humans. A cDNA library from a male *Macaca fascicularis* liver was constructed in Uni-λZAP XR vector and screened using the human alpha-class GST-A1 cDNA as a probe. Five positive clones were plaque purified. Restriction mapping and sequence analyses revealed the presence of three different full-length cDNA clones. These GST clones were highly homologous to human alpha-class GST A1. One of the three clones comprises 992 bp, 669 bp of which encode a protein of 222 amino acids that shares 93% homology with human A1. Currently, we are characterizing these cDNAs and the corresponding proteins with respect to their AFBO activities. (Supported by ES-05780 and partially by RR00166).

1453 THE EFFECT OF PROBENECID, VANADATE, AND VERAPAMIL ON EXTRUSION OF THE GLUTATHIONE CONJUGATE OF MONOCHLOROBIMANE BY RAT-1 FIBROBLASTS

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The glutathione-s-transferase substrate monochlorobimane has been used to measure glutathione content in individual cells by flow cytometry and image cytometry. MCB is nonfluorescent, but when conjugated to GSH by GST, the resulting GS-bimane conjugate is highy fluorescent. We have previously reported that the GS-bimane conjugate can be extruded from rat-1 fibroblasts, and this can be inhibited by the organic anion pump inhibitor probenecid. However, it has also been reported that some GSH conjugates can be transported out of cells by a vanadate inhibitable (ATP-dependent) transporter. Additionally, multidrug resistance in tumor cells is often associated with an increase in p-glycoprotein 170 (p170; MDR-1) which is inhibited by verapamil. We therefore examined the effectiveness of vanadate and verapamil on the extrusion of GS-bimane from rat-1 fibroblasts compared to probenecid using flow cytometry. Verapamil was able to partially inhibit the extrusion of GS-bimane at relatively high concentrations (100 μM), whereas vanadate actually increased the loss of fluorescence compared to controls. As expected, probenecid (1–2 mM) significantly retarded the efflux of GS-bimane compared to untreated controls. These data suggest that the principal route of GS-bimane from rat-1 fibroblasts is via a probenecid-inhibitable organic ion transport pump. This work was supported by NIH grants ES04696 and AG01751.

1454 VALIDATION OF A METHOD FOR THE DETERMINATION OF GSH, GSSG, AND SULFER CONTAINING AMINO ACIDS USING HPLC-EC

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A number of methods are available for the determination of glutathione, GSSG and sulfur containing amino acids in cells. Derivitive formation with monobromobimane is frequently used, but while the method is sensitive, it is incapable of monitoring disulfides such as GSSG. We report the rapid, sensitive and specific method for determination of GSH and GSSG in small samples of lung tissue microdissected from mice. Tissue is homogenized in methane-sulfonic acid, centrifuged and the supernatant is injected onto an HPLC column. HPLC conditions were as follows G = 0.95V, E1 = +0.4V, E2 = +.875V; 1 ml/min, 2% ACN in 50 mM NaH2PO4, 0.05 mM octane sulfonic acid; pH 2.7; with a Phase-Sep ODS2 column. Limit of detection of GSH and GSSG were 0.4pmol and 1.2pmol respectively. Intrassay coefficient of variation for the determination of GSH and GSSG was 4.7 and 4.4% respectively in the trachea, 5.9 and 5.7% in the parenchyma. Quantities of GSH and GSSG in microdissected airway trees and parenchyma were in the 200–300 pmol and 12–16 pmol and 100–120 pmol and 10–20 pmol ranges respectively. These conditions were able to easily resolve pmol quantities of cysteine, methionine, cys-gly, cys-SG. Supported by ES05578, ES04311, ES00628, ES06700.

1455 MECHANISMS OF ACROLEIN MERCAPTURATE TOXICITY

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The ability to form acrolein is necessary for the toxicities associated with cyclophosphamide (CP). Acrolein, 3-oxopropyl glutathione (oxoPrGSH), and the mercapturic acid S-3 hydroxypropyl N-acetyl-cysteine (hydroxyPrMCA) are confirmed metabolic products of CP. Other potential metabolites include S-3 oxopropyl N-acetyl-cysteine (oxoPrMCA), its sulfoxide and the corresponding diacid forms. The reactivity of acrolein would appear to preclude its movement from the main site of formation (liver) to the sites of toxicity (lung, bladder). The re-release of acrolein from various thiol conjugates via a β-elimination reaction or the direct toxicity of these conjugates are possible mechanisms to explain the toxicities of CP. The relative toxicities of oxoPrMCA and its S-oxide to human lung adenocarcinoma A549 cells correlated with their abilities to release acrolein. Although depleting GSH by pretreatment with diethyl maleate enhanced the toxicity of oxoPrGSH (24 h treatment), oxoPrMCA and its S-oxide (2 h treatments), and exogenous GSH provided protection from toxicity, depletion of intracellular GSH only occured at doses above those needed to significantly inhibit cell proliferation. The diacid conjugate showed no toxicity to A549 cells, indicating further oxidation products of the mercapturic acids are not a factor. ButanoneMCA, an analog of oxoPrMCA which in contrast to oxoPrMCA cannot exist in the geminal diol form, inhibited A549 cell growth only slight less effectively than oxoPrMCA, suggesting the geminal diol is not toxicologically significant. OxoBuMCA, which cannot undergo β-elimination of acrolein, showed no toxicity to these cells, suggesting that the release of acrolein is critical. The protective effect of extracellular GSH and the decreased fluorescence seen in cells treated with acrolein then exposed to *p*-sulfobenzoyloxybromobimane, a non-membrane permeable thiol probe, suggest conjugate toxicity was mediated via an extracellular site of action. (Supported by HL48035).

1456 EFFECT OF THE PYRROLIZIDINE ALKALOID, MONOCROTALINE, ON BILE COMPOSITION IN RAT

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Sponsor: A J Gandolfi

The pyrrolizidine alkaloid, monocrotaline, is bioactivated in the liver to the hepatotoxic metabolite, dehydromonocrotaline. The effect of monocrotaline exposure on bile composition *in vivo* has not been examined. Sprague-Dawley rats (Body weight 250–300) were divided into two groups of 5 animals. One group was injected i.p. with monocrotaline (65 mg/kg) and the other group with an equivalent volume of saline. After 24 hr, the rats were anesthetized and the bile duct was cannulated with polyethylene tube (PE 10). Bile was collected for 1 hr into preweighed microfuge tubes placed in ice. In monocrotaline-treated rats, bile flow and biliary concentration of lipids (bile salts, phospholipids and cholesterol) were significantly depressed. Cholesterol concentration fell from 0.71 mM in control rats to 0.42 mM in monocrotaline-exposed rats. The biliary concentration of taurine also fell, from 0.30 mM to 0.17 mM. Biliary excretion of cysteine, cysteinylglycine and γ-glutamylcysteine, on the other hand, was significantly increased in the monocrotaline-exposed group. Cysteine, for example, increased from 0.18 mM in control to 0.38 mM in monocrotaline-exposed rats. These findings suggest that monocrotaline disturbs both lipid and sulfur metabolism in the liver. As sulfhydryl compounds are involved in the detoxification of monocrotaline, it appears that there is a mutual interaction of sulfur metabolism and monocrotaline metabolism. Partially supported by USPHS HL 25258 and SWEHSC P30-ES-06694

1457 *IN VIVO* EFFECT OF CADMIUM, COPPER AND LEAD ON GLUTATHIONE CONCENTRATION IN MERCENARIA BROWN CELLS

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The intent of this study was to test the hypothesis that Cd^{2+} cause depletion of glutathione (GSH) in brown cells of *Mercenaria mercenaria* (Bivalvia). Brown cell involvement in detoxification is due tin part to endogenous GSH which conjugates with many divalent metals and xenobiotics. When compared to controls, *Mercenaria* injected with 20 mM Cd^{2+}, 1.0 mM Cu^{2+} or 5.0 mM Pb^{2+} had significantly less GSH in brown cells. Lower GSH concentrations due to each metal treatment was not dose dependent. However, N-methylmaleimide (NEM), which binds irreversibly to sulfhydryl groups and used as a positive control in this study, lowered brown cell GSH concentrations in a dose dependent manner after injection into *Mercenaria*. When the fluorescent probe monochlorobimane (mBCI) which is specific for GSH was used in conjunction with laser cytometry, it became apparent that the fluorescent conjugate was diffusely distributed in the cytoplasm and is heterogeneously distributed among brown cell populations. In addition, mBCI treatment of brown cells from *Mercenaria* injected with Cd^{2+}. Cu^{2+} or Pb^{2+} indicated that brown cell resistance to metal cytotoxicity may be due to interference with metal transport across brown cell membranes. These results indicate that partial not complete depletion of GSH with metals is possible in brown cells.

1458 *IN VIVO* FORMATION OF 5-(GLUTATHION-S-YL)-α-METHYLDOPAMINE FOLLOWING ADMINISTRATION OF METHYLENEDIOXYAMPHETAMINE: EVIDENCE FOR THIOL-CONJUGATE BRAIN UPTAKE

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5-(Glutathion-S-yl)-α-methyldopamine (5-GSyl-α-MeDA) is a putative metabolite of the serotonergic neurotoxicant methylenedioxyamphetamine (MDA). Previously we proposed a role for 5-GSyl-α-MeDA in the acute and long term neurochemical changes observed after administration of MDA and demonstrated that the brain has the ability to synthesize mercapturic acids following intracerebroventricular (icv) administration of 0.72 μmol 5-GSyl-α-MeDA to Sprague-Dawley rats. Additionally, icv administration of 5-GSyl-α-MeDA produced behavioral changes which paralleled those observed following subcutaneous administration of MDA. To demonstrate *in vivo* formation of 5-GSyl-α-MeDA, we collected bile following administration of MDA (23 μmol; sc). The total amount of 5-GSyl-α-MeDA recovered during the 5 hour period was 378 ± 62 nmoles, which represents 1.6% of the administered dose of MDA. Additionally, evidence for brain uptake of 5-GSyl-α-MeDA was obtained utilizing the intracarotid artery administration technique of Oldendorf. The brain uptake index (BUI) for 5-GSyl-α-MeDA (8.5 μM) was 7.4 ± 0.5% and was increased to 48.8 ± 3.8% by pretreatment with acivicin. Coinjection of 1 mM GSH with or without acivicin pretreatment decreased the BUI to 2.5 ± 0.8% and 4.0 ± 0.9% respectively. These data demonstrate the peripheral formation and brain uptake of 5-GSyl-α-MeDA and support the idea that 5-GSyl-α-MeDA may be involved in the acute neurochemical and behavioral changes seen after administration of MDA.

1459 SIMULTANEOUS DETECTION OF GSTM1 AND GSTT1 DELETION-TYPE POLYMORPHISMS USING A MULTIPLEX POLYMERASE CHAIN REACTION (PCR) ASSAY

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Glutathione S-transferase (GST) biotransformation enzymes catalyze conjugation reactions of glutathione (GSH) with electrophilic compounds, including known carcinogens. GSTM1 has been implicated in the detoxification of dietary nitrosamines and benzo[a]pyrene, while GSTT1 conjugates monohalomethanes and ethylene oxide. GSTM1 and GSTT1 have deletion-type polymorphisms. Due to the interest in examining genetic predisposition to environmentally-induced carcinogenesis, we have developed a multiplex **PCR** assay that detects polymorphisms in both of these GSTs simultaneously. Since both are deletions, amplification of the β-globin gene is included as an internal control. The protocol requires a hot-start **PCR** procedure with buffer containing 10mM Tris-HCl, 2.0mM MgCl2, 50mM KCl at pH 9.2; 2.0nM of each **GSTM1** and β-globin primers (**JNCI**, 84:14,1159–64); 1.0nM of **GSTT1** primers (Cancer Epi, Biomarkers & Prevention, 4, 253–9); and 25ng to 100ng of DNA. 40mM dNTPs and 1.5 units Taq DNA polymerase are added just prior to the completion of the initial denaturation step. Amplification is performed on the Hybaid Omnigene thermocycler with 1 cycle of 93°C for 4 minutes, 60°C for 45 seconds, and 72°C for 90 seconds, followed by 40 cycles of 93°C, 61°C, and 72°C for 30, 45, and 90 seconds, respectively. Ongoing experiments are being performed to allow for quantitation of GSTM1 and possibly GSTT1 by optimizing PCR conditions to the linear range of amplification or use of a recombinant DNA internal standard. Results from these studies may lead to a genotyping assay to differentiate homozygous positive and hemizygous individuals and will be useful in large-scale molecular epidemiological studies. (Supported by NIEHS Grant 1P30ESO7033–01).

1460 TRANSFECTION AND EXPRESSION OF THE MOUSE cDNA ENCODING THE CATALYTIC AND REGULATORY SUBUNITS OF GLUTAMYLCYSTEINYL LIGASE IN MAMMALIAN CELLS

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Glutamylcysteinyl ligase (GLCL), formerly known as gamma glutamylcysteine synthetase, is the first and rate limiting enzyme in the synthesis of glutathione (GSH). Using RT-PCR, the open reading frames for both the regulatory (GLCLR) and catalytic (GLCLC) subunits of mouse GLCL were isolated and subcloned into the mammalian expression vector pCRTM 3-UNI. Expression vector construct activity was verified *in vitro* using Novagen's Single Tube ProteinTM System 2,T7. COS-1 cells were transfected and analyzed for both transient and stable alterations in GLCL expression and GSH levels. Both transiently and stably transfected cells were found to have increased GLCLR and GLCLC mRNA levels and enzymatic activity. GSH depletion/repletion studies demonstrated increased ability of transfected cells to replenish GSH. These cells also demonstrated higher rebound GSH levels when compared to control cells. In summary, we have successfully cloned and transfected mammalian cells with both the regulatory and catalytic subunits of GLCL and studies are underway to evaluate the protective role of GSH upregulation. Supported by NIH grants AG01751, ES04696, and AG00057.

1461 GLUTATHIONE S-TRANSFERASES (GST) IN INTESTINAL AND HEPATIC CYTOSOL FROM CHANNEL CATFISH

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Previous studies showed that dietary benzo(a)pyrene (BaP) was readily biotransformed in the intestine of control and β-naphthoflavone (BNF)-induced channel catfish to several metabolites including glutathione (GSH) conjugates. Overall biotransformation of BaP was higher in induced catfish, although at the doses of BNF used, there was no effect on GST activities with 1-chloro-2, 4-dinitrobenzene (CDNB) in intestinal cytosol. The apparent K_m values for CDNB with intestinal and hepatic cytosol from control catfish were 0.97 ± 0.19 mM (mean ± S.D., n=5) and 0.85 ± 0.17 mM respectively. The V_{max} values for intestine and liver were 3.29 ± 0.53 and 4.54 ± 0.85 μmol/min/mg protein, respectively. GST was purified from dialysed intestinal mucosal cytosol and hepatic cytosol of control catfish by anion exchange chromatography with DE-52 resin (liver only) and affinity chromatography with GSH-agarose. By following major peaks of activity with CDNB only, an 18 fold purification of hepatic GSTs was achieved. The intestinal GSTs that bound to the GSH-agarose were purified 32 fold from cytosol. In the absence of GSH or dithiothreitol, intestinal GST was unstable. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the fractions from affinity chromatography revealed the presence of major bands with molecular weights 26,500 in the liver, and 27,000 in the intestine. The intestine also had a minor band at molecular weight 30,000. Isoelectric focusing showed that the major intestinal GSTs were acidic. This study re-emphasizes the role of the intestine in first pass biotransformation of dietary xenobiotics. Supported in part by US PHS, ES 05781.

1462 EFFECT OF CURCUMIN ON GLUTATHIONE (GSH) LEVELS AND GSH RELATED ENZYMES IN RAT LIVER

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Curcumin, a constituent of the commonly used spice turmeric (*Curcuma longa* Linn.), has been shown to inhibit tumor formation in three different models of chemically induced carcinogenesis in rodents (Huang *et al.*, Cancer

Res. 54:5841–5847, 1994). Since the induction of GSH-linked detoxification systems has been suggested to be one of the mechanisms for the anticarcinogenic activity of a number of chemoprotective agents, present studies were designed to examine the effect of curcumin on GSH levels and GSH related enzymes in rat liver. When rats were fed with 75, 250, and 500 mg curcumin/kg body weight/day for two weeks, no significant differences were observed in total cytosolic glutathione S-transferase (GST) activity towards 1-chloro-2,4-dinitrobenzene between control and treated rats. Furthermore, no significant differences were found in the amount of total GST protein in the liver of control and treated animals. Separation and quantitation of individual GST isozymes, however, indicated a significant increase in rGST 8–8 which is known to use the endogenous products of lipid peroxidation (e.g. 4-hydroxyalkenals and lipid hydroperoxides) as preferred substrates. Western blot analyses of total GSTs showed an approximately 2-fold induction of rGST8–8 at the 75 mg/Kg dose level. No noticeable increase was observed in GST proteins within the α and μ classes, which constitute the majority of rat liver GST protein, which was consistent with the findings that total GST activity was not affected by curcumin. Curcumin treated rats showed maximal induction of glutathione peroxidase activity and γ-glutamyl cysteine synthetase activity at doses of 75 mg/Kg and 250 mg/Kg, respectively. The results of these studies suggest that curcumin treatment modulates GSH-mediated antioxidant defense mechanisms, and this, in conjunction with the inherent antioxidant properties of curcumin, may account at least in part for the observed chemoprotective effect of curcumin against chemical carcinogenesis. (CA-27967 and CA-63660)

1463 MYRISTICIN INDUCES GLUTATHIONE TRANSFERASE ISOENZYMES IN MOUSE TISSUES

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Myristicin (4-methoxy-6-[2-propenyl]-1,3-benzodioxole) [607-91-0], an active constituent of parsley (*Petroselinum sativum* Hoffm.) leaf, is a known antiinflammatory and chemopreventive agent. We have investigated its potential to induce glutathione S-transferase isoenzymes (GST; EC 2.5.1.18) as a mechanism for its chemopreventive action. The three major classes of GST (α, μ, π), are involved in the detoxification of electrophilic xenobiotics including carcinogens. Oral administration of myristicin at 5 and 20 mg / mouse (albino A/JOlaHsd) selectively and differentially induced specific GST isoenzyemes in tissues investigated. The substrate specificity and western blot data show that, in liver, one of the two GST μ isoenzymes and GST α isoenzyme were significantly induced. In lung only the GST π was slightly induced, whereas in brain and kidney there was a slight increase in GST μ and GST π isoenzymes. Myristicin at 50 mg / mouse was toxic to all tissues studied. These findings suggest that the anticarcinogenic activity of myristicin may be mediated through GST induction. (Supported in part by NIH grants GM50080 and SO6GM08038).

1464 DITHIOTHREITOL PREVENTS CA^{2+} AND TERT-BUTYLHYDROPEROXIDE INDUCED OXIDATION AND RELEASE OF GLUTATHIONE AND PYRIDINE NUCLEOTIDES FROM ISOLATED RAT LIVER MITOCHONDRIA

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Mitochondrial glutathione (GSH) status plays an important role in maintaining the viability of cells exposed to oxidative stress. Addition of Ca^{2+} and 200μM tert-butylhydroperoxide (TBH) to isolated liver mitochondria caused a rapid and complete oxidation of GSH, subsequent formation of glutathione protein mixed disulfides, and release of GSH and GSSG glutathione from the matrix through induction of the permeability transition pore. The loss of glutathione was accompanied by rapid oxidation and subsequent release of pyridine nucleotides through the pore. Addition of the thiol reducing agent dithiothreitol (DTT) restored the integrity and redox status of the mitochondria. Our results demonstrate that in the presence of external reducing equivalents, mitochondria maintain their glutathione status which protects them from oxidative stress to a greater degree than cyclosporin A (0.5μM). Maintenance of mitochondrial functional integrity may provide a crucial cytoprotective role under conditions of oxidative stress. This work was supported by NIH Grants ES-O1978 and ES-O0210.

1465 ENHANCED GLUTATHIONE SYNTHESIS CAPACITY IS A MECHANISM FOR CADMIUM/ADRIAMYCIN CROSS RESISTANCE IN HUMAN LUNG CARCINOMA A549 CELLS

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Cross resistance presents an obstacle in cancer chemotherapy. Cadmium (Cd) is a potential carcinogen and its exposure has resulted in lung cancer. Cd also induces resistance in human lung carcinoma cells. This resistance may be shared by antineoplastic agents, which needs to be considered for chemotherapy of Cd-induced lung cancer. In the present study, two subpopulations of human lung carcinoma A549 cells with a different magnitude of resistance to Cd were shown to have a parallel resistance to Adriamycin (ADR), an important anticancer drug. Several factors were examined to determine the mechanism(s) for the cross resistance, including cellular metallothionein and glutathione (GSH) concentrations, glutathione S-transferase activity, MDR1 expression, and antioxidant enzyme activities including superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase. Only cellular GSH content was consistently elevated in the Cd/ADR resistant cells. Other factors were not significantly different between the Cd/ADR resistant and sensitive cells. Treatment with buthionine sulfoximine, a specific inhibitor of GSH synthesis, depleted GSH contents and reversed the resistance to both Cd and ADR in the resistant cells. Further studies showed that overexpression but not amplification of γ-glutamylcysteine synthetase (γ-GCS), which catalyzes the rate-limiting step in de novo GSH synthesis, is responsible for the elevated GSH levels. The results demonstrate that cross resistance to ADR exists in Cd-resistant human lung carcinoma A549 cells. Enhanced GSH synthesis capacity is a mechanism for the cross resistance. Supported in part by NIH Grants CA68125 and CA63752.

1466 A GENETIC SCREEN FOR MUTATIONS IN THE YEAST YAP1 SIGNAL TRANSDUCTION PATHWAY OF OXIDANT STRESS RESPONSE

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Sponsor: R Snyder

The control of the cellular redox milieu is achieved by an array of antioxidant scavenging activities whose levels are set according to changes in intracellular redox conditions. In prokaryotes, the redox sensors OxyR and SoxR alert the bacterial chromosome of any redox perturbation, thereby leading to a cellular adaptive response directed toward the correction of the initiating perturbation. Eukaryotic cells are also able to adapt to redox perturbations but the mechanisms of detection and transmission of the oxidant signal are poorly understood. We have used *S. cerevisiae* as a model organism to study the eukaryotic signal transduction pathways of oxidative stress responses. The yeast mammal AP1 equivalent YAP1 controls the expression of *TRX2*-encoded thioredoxin 2 and of *GSH1*-encoded γ-glutamyl cysteinyl synthase, both important antioxidant defense mechanisms. YAP1 is activated by H_2O_2 but the pathways transducing the oxidant signal to this transcriptional regulator are not elucidated. We have created recombinant strains carrying a gene fusion placing the URA3 gene marker under transcriptional control of YAP1. These strains are URA3 conditional auxotrophs and have been used in a genetic screen for search of constitutive or uninducible mutations in the YAP1 signal transduction pathway. We will present the characterization of these mutations.

1467 ELECTROPHILE REGULATION OF GENES IN TWO MURINE CELL LINES

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Cultured hepatocytes from newborn mutant C^{14CoS}/C^{14CoS} mice (*14CoS* cells) have higher levels of glutathione (GSH), NAD(P)H: menadione oxidoreductase (NMO1) and aldehyde dehydrogenase (AHD4), compared with hepatocytes from wild-type c^{ch}/c^{ch} mice (*ch* cells), possibly due to deletion of the fumarylacetoacetate hydrolase (*Fah*) gene, blocking tyrosine degradation and generating electrophilic intermediates. Such electrophiles up-regulate NMO1 and AHD4 by interacting with an electrophile response element (EpRE), known to be located in the 5'-flanking region of *Nmo1* and *Ahd4*. The rate-limiting enzyme in GSH biosynthesis, γ-glutamylcysteine synthetase (GCS), is less understood. Exposure to the electrophile, λbutylhydroquinone (tBHQ), increased NMO1, AHD4, GCS, and GSH levels in both *14CoS* and *ch*

cells. Increases in enzyme activities and GSH levels were accompanied by corresponding elevation of NMO1, AHD4 and GCS mRNA levels. However, maximal levels of induction by tBHQ in *14CoS* cells was significantly greater than those obtained in *ch* cells. These data indicate that these cell lines provide a suitable model for determining factors involved in regulation of constitutive and inducible levels of GSH, and genes under the control of an EpRE. (Supported by NIH T32-ESO7278, P30-ESO6096 and RO1-AGO9235).

1468 THE ROLE OF HEPATIC GLUTATHIONE IN CIRCADIAN VARIATION OF ACETAMINOPHEN HEPATOTOXICITY

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Experiments were conducted to examine the circadian variation in acetaminophen (APAP) hepatotoxicity using male ICR mice. Animals were injected with a single dose of APAP (400 mg/kg, ip) at either 8 am, 2 pm or 8 pm of a day, and the hepatotoxicity was determined 24 hrs following each treatment. Significant increases in hepatotoxicity were observed in the mice treated with APAP at 8 pm compared to those treated at 8 am or 2 pm as shown by elevations in serum alanine aminotransferase and aspartate aminotransferase activity. Decreases in glucose-6-phosphatase activity in liver were greatest in the 8 pm group. Hepatic GSH levels in mice were highest at 8 am and were decreasing at 2 pm and 8 pm. In animals maintained with a liquid diet containing 5 % (w/v) glucose for 24 or 48 hrs, the hepatic GSH levels reduced to approximately a half of the control value. However, the circadian variation in hepatic GSH levels was abolished in these animals indicating that hepatic GSH levels are closely correlated with diet consumption. Differences in the hepatotoxicity among mice treated with APAP at different times of the day were markedly diminished but not completely disappeared. The results in the present study indicate that the circadian variation in APAP hepatotoxicity is dependent mostly, but not totally, on the changes in hepatic GSH levels induced by food intake. (Supported by a RCNDD grant 95-K3-16 35-6-2-1 from KOSEF)

1469 STRUCTURE FUNCTION ANALYSIS OF AFLATOXIN B1-EPOXIDE CONJUGATING ACTIVITIES IN GLUTATHIONE S-TRANSFERASE-YC ISOENZYMES

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Mice constitutively express glutathione S-transferase (GST) mYc in liver which possesses high conjugating activity toward aflatoxin 8, 9-epoxide (AFBO) protecting them from aflatoxin B1 (AFB)-induced hepatocarcinogenicity. In contrast, the constitutively expressed homologous rat isoform (rYc_1) has low AFBO conjugating activity, and as a result rats are sensitive even to low levels of AFB exposure. Although the two GSTs share 86% sequence identity and have similar activity towards CDNB, the AFBO conjugating activity of the rat Yc_1 enzyme is less than 1% of the mouse enzyme. In order to identify amino acids that confer high activity toward AFBO, rYc_1 residues were replaced with the corresponding mYc residues in two non-conserved regions of rYc_1 around the enzyme active site by site-directed mutagenesis. Residues were selected for mutagenesis based on sequence alignment with the human A1 GST and analysis of a computer generated model of mYc derived from the human A1 crystal structure. Wild-type mYc and rYc_1 had AFBO conjugating activities of 183.6 and 0.3 nmol/mg/min, respectively. As an extension to previous site-directed mutagenesis work, we have added an additional mutation at residue 217 (V to K). The single site mutant V217K exhibited no detectable AFBO activity, but when combined with previous mutation sites (residues 104, 108, 207, and 208) the mutation increased activity by 2.5 fold. The rYc_1 mutant carrying five mutations (residues 104, 108, 207, 208, and 217) had an AFBO activity of 7.5 nmol/mg/min, a 25 fold increase over the wild-type rYc_1.

1470 CHLOROFORM-INDUCED GLUTATHIONE DEPLETION AND CYTOLETHALITY IN FRESHLY ISOLATED MOUSE HEPATOCYTES

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Chloroform-induced hepatocarcinogenesis is believed to be secondary to cell proliferation and other events associated with cytotoxicity. Understanding the mechanisms of chloroform toxicity may provide insights into the mechanisms of mutation induction that leads to cancer. Freshly isolated B6C3F1 male mouse hepatocytes in suspension were exposed to chloroform and then cultivated for 24 hr as monolayers to observe cytolethality. The exposure concentrations (0.6–3.8 mM) and durations (1–3 hr) chosen were based on the exposure of the liver *in vivo* after hepatotoxic doses of chloro-form. Following 2-hr exposure, chloroform induced cyto-toxicity (LDH release) in culture at concentrations > 1 mM. The same threshold was found for glutathione (GSH) depletion, with a 50% depletion at 3.8 mM after 2 hr. However, no significant protein sulfhydryl group depletion was observed. Addition of dithiothreitol did not prevent toxicity, indicating that oxidation of sulfhydryl groups is not critical for toxicity. Phenylimidazole cotreatment totally prevented both cytolethality and GSH depletion, indicating that metabolism is necessary for chloroform-induced toxicity. Since chloroform metabolism is saturated in the micromolar range, our results indicate that both metabolism and exposure of the cells to high concentrations of chloroform are required for toxicity.

1471 ISOLATION OF THE HUMAN MICROSOMAL GLUTATHIONE TRANSFERASE GENE (GST12) AND CHARACTERIZATION OF THE PROMOTER REGION

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A P1 clone containing the GST12 gene was isolated from a human P1 phagemid library. The gene contains 4 exons and spans 14.5 kb. Flanking 5' and 3' regions were sequenced and exon/intron boundaries defined. Exon sequencing revealed 2 nucleotide differences from a previous report on the cDNA sequence. The substitutions were silent as they did not alter amino acid composition. In agreement with a previous report on GST12 cDNA sequence, an in-phase stop codon and an out-of-phase false start codon were present in the 5' untranslated region. If a minicistron is present reinitiation at a downstream AUG can occur. A second start codon, however, for the in-phase stop codon could not be identified in the early 5' untranslated region nor for > 300 bp in the promoter region. Thus, GST12 expression violates the first-AUG rule. According to the ribosomal scanning model for eukaryotic translation initiation, leaky scanning can occur if the first AUG codon exists in suboptimal context (lacks Kozak consensus sequence). As the first start codon lacks the critical G at the +4 position, this must be occurring with GST12 expression. Supported by NIH ESO49889 & CA52310.

1472 IMPACT OF BENZO(A)PYRENE AND RELATED AROMATIC HYDROCARBONS ON HEPATOCYTE GLUTATHIONE LEVELS

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The toxic effects of benzo(a)pyrene (BaP) and related aromatic hydrocarbons (AHs) may involve interference with cellular antioxidant mechanisms leading to oxidative stress. In the present study, the impact of selected AHs on hepatocyte glutathione (GSH) levels was evaluated to determine if class-specific differences in the modulation of GSH exist for this group of chemicals. Hepatocytes were treated with benzo(a)pyrene (BaP, 0.3–30 μM), 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD, 0.0001–1 nM), 2,3,7,8-tetrachlorodibenzofuran (TCDF, 0.005–5 nM), naphthalene (NAPH, 0.1–1000 μM), 2-methylnaphthalene (2-MNAPH, 0.1–1000 μM), or pentachlorophenol (PCP, 0.1–1000 μM) for various times and processed for measurements of GSH levels. Marked increases of hepatocyte GSH levels were observed in hepatocytes treated with BaP (0.3–30 μM) for 24 hr. Only modest increases in GSH levels relative to controls were observed in cultures treated with TCDD (0.001–1 nM) or TCDF (0.005–5 nM) for 24 hr. Time-course studies revealed that upregulation of cellular GSH by BaP and TCDD was preceded by depletion of GSH as early as 6 hr following treatment. NAPH decreased hepatocyte GSH levels transiently at 4–16 hr. Based on these data we conclude that hepatocytes exhibit class-specific differences in the modulation of GSH by AHs. (Supported by NIH grants ES04917 and ES00213).

1473 ANDROGEN EFFECT ON γ-GLUTAMYLTRANSPEPTIDASE (GGT) OF HUMAN PROSTATE CANCER CELLS

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Androgens such as testosterone and dihydrotestosterone play a crucial role in both normal prostate growth and development and in prostate carcinogenesis. Androgen modulation is often used therapeutically to treat prostate cancer. We have studied the effect androgens have on the glutathione (GSH) detoxification system of prostate cells using androgen dependent, LNCaP, and andro-

gen unresponsive, DU145, prostate carcinoma cell lines. Physiologically relevant levels of the synthetic androgen, R1881, have significant effects on GGT in LNCaP but not DU145 cells. GGT activity of LNCaP cells is elevated 2-fold above control 48-96h after treatment. Serine borate, a competitive inhibitor of GGT, blocks the R1881 stimulated increase in GGT activity. Immunohistochemical staining for GGT shows an increase in GGT-positive staining in LNCaP cells treated with 1nM R1881. Steady state GGT mRNA levels are also increased at this dose. In correlation with the increase in GGT activity, cells pretreated with 1nM R1881 display a greater rate of loss of GSH from the media, increased oxidative stress and lipid peroxidation, and a slower rate of growth. Serine borate blocks this increase in oxidative stress as well as dampening the effect of R1881 on LNCaP cell growth.

1474 STRUCTURE ACTIVITY RELATION ON THE INTERACTION OF ACRYLONITRILE (VCN) AND METHACRYLONITRILE (MeVCN) WITH GLUTATHIONE

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MeVCN, an α methyl analogue of VCN is being considered in the polymer industry. Recent studies indicated that the slight structural difference between these two compounds imparted significant differences in their disposition and biliary excretion. Glutathione (GSH) and glutathione transferase (GSTs) play an important role in the conjugation and biliary excretion of electrophilic substances. To clarify the mechanism leading to the marked difference in the disposition of these chemicals, we examined the structure activity relationship of VCN and MeVCN in their direct and enzymic interaction with GSH. Variable concentrations of VCN or MeVCN were incubated (37°C, pH-8.0) with GSH with or without rat hepatic cytosol. GSH was determined by Ellman's method. The results indicated that VCN reaction with GSH is concentration and time dependant and followed Michael's addition reaction mechanism. The rate of VCN reaction with GSH was faster (450%) than that of MeVCN. Reaction of MeVCN (ImM) with GSH was minimal till 2 h. of incubation. Addition of rat hepatic cytosolic GST to the incubation mixtures catalyzed the conjugation of both compounds with GSH. There was, however, a 5 fold increase in the rate of VCN reaction with GSH in contrast to the of MeVCN. The lower electrophilicity of MeVCN incited by the α methyl substitution and the consequent slow neucleophilic addition as compared to VCN play a role in the observed differential reactivity towards GSH and may explain the reported difference in their biologic fate. [Supported by NIEHS Grant No. ES 01871].

1475 DISTRIBUTION OF GLUTATHIONE *S*-TRANSFERASE CLASS PI AND ALPHA ISOZYMES IN URETHANE-INDUCED LUNG TUMORS

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The distribution of the glutathione *S*-transferase (GST) isozymes, class pi and alpha, was investigated in urethane-induced lung tumors under control conditions and after treatment with butylated hydroxyanisole (BHA). Western blotting detected a single protein in cytosol migrating at ~28 kDa for pi and ~29 kDa for alpha. Treatment with BHA induced alpha to a greater extent than pi. Immunohistochemical studies showed that, in control lung, immunoreactivity for GST alpha was greater than that observed for the pi class. Staining for both alpha and pi was highly localized in the Clara cells and to a lesser extent in endothelial and alveolar type II cells. Staining levels for alpha were similar in hyperplastic foci and papillary tumors, and were both higher than in control lung. The amounts of staining for pi were substantial in hyperplastic foci and solid tumors, and low in papillary tumors, but were all higher than in normal lung. Treatment of nontumor- and tumor-bearing mice with BHA did not produce induction of either alpha or pi in tumor foci; however, induction of alpha was evident in Clara cells of bronchioles. These results demonstrated that the expression of alpha and pi is higher in tumors than in normal lung, and is not inducible by BHA. Supported by MRC of Canada.

1476 CHANGES' PECULIARITIES OF GLUTATHIONE POOL IN RATS LIVER BY ACTION OF HEPATOTOXINS OF DIVERSE NATURE

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Many industrial compounds to which number CCL_4 and thioacetamide (TAA) are concerning have a hepatodepressive effect owing to damage in cellular chemical homeostasis. Glutathione system is the factor responsible for resistance to toxicants. Studies this system in liver of rats undergone single subcutaneous administration by thioacetamide (20 mg/100g) illustrated increase of total reduced (GSH) and especially oxidized (GSSG) (2, 77 times) on the first day. In 4 days after administartion of hepatotoxin it was revealed the increase of all pool of glutathione, GSSG increasing 7, 72 times, GSH - only 47%. On the days 7 and 10 the level of GSSG was lowering gradually, but was 2, 12 and 1, 80 times the control indices. GSH was increasing 103, 8 and 90, 0%. Compared with TAA CCl_4 has increased sharply the level of GSSG only on the 1.day, then its content neared gradually control indices, but content of GSH had no essential changes. Differences revealed are caused by various mechanisms of destructive action of studied hepatotoxins, that should be taken into consideration in industrial production.

1477 LONG TERM ASSESSMENT OF AN ACCIDENTAL EXPOSURE TO POLYCHLORINATED BIPHENYLS IN A RHESUS MONKEY COLONY

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Congener specific analysis of polychlorinated biphenyls (PCBs) in rhesus monkey serum was performed as a follow up to a 1982 report (Lab. Anim. Sci. 32:83, 1982) describing PCB poisoning which occurred in a rhesus monkey colony at the Bowman Gray School of Medicine primate facility. The animals involved in this study provide a unique opportunity to examine potential effects of PCBs since this population had undergone chronic exposure from contaminated paint chips until the suspected source of contamination was abated. The samples analyzed were obtained more than ten years after the initial exposure had ceased. Congener profiles differed from that typically seen in humans. Congener 180 was the most prevalent congener identified, exceeding the levels of 153 and 138 in most animals. Animals with the highest levels of congener 180 had either lived in the contaminated facility, been born there, or had mothers who had lived in the facility. Mothers having greatest risk for exposure experienced a dramatic increase in the number of stillbirths, when compared to mothers living in the facility after the PCB problem had been addressed. Of six separate groups of monkeys in the facility, oophorectomized females receiving 1,7-dihydroequilin and untreated males had the highest PCB levels. The samples that have been analyzed to date were collected in 1994 at necropsy. Banked serum samples from the same monkeys taken in 1990 offer the opportunity to examine the half-lives of specific PCB congeners in this unique population.
This work was funded in part by EPA contract number 4D2392NTEX.

1478 CYTOTOXICITY OF DDT METABOLITES TO IMMORTALIZED HUMAN BRONCHIAL EPITHELIAL CELLS

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The high susceptibility to DDD of rabbit Clara cells and a transformed human bronchial cell line has been reported by our laboratory (Drug Metabolism and Disposition, 23, 595, 1995). BEAS-2B is a transformed human bronchial epithelial cell line that has characteristics of normal human bronchial epithelial cells, including enzymes for bioactivation and detoxification of recognized carcinogens. The current study assessed the cytotoxicity of five DDT metabolites using BEAS-2B cells and isolated rabbit Clara cells and alveolar macrophages. 1,1-dichloro-2,2-bis (p-chlorophenyl)ethane (DDD) and the dichloroethene metabolite, DDE, are stable metabolites of DDT that induce tumors in liver and lung, and undergo metabolism via the chloro-olefin, DDMU. DDD is cytotoxic to BEAS-2B cells and rabbit Clara cells and alveolar macrophages at concentrations as low as 0.01mM. DDMU and DDE were also cytotoxic, although at much higher concentrations (0.1 and 0.5 mM). The de-chlorinated ethane metabolite, DDNU, a hydroxylated metabolite, DDOH, and the fully oxidized acetic acid metabolite, DDA, were not cytotoxic. The highly selective cytotoxicity of DDD for Clara cells and BEAS-

2B cells is correlated with a cytochrome P450-mediated bioactivation. In contrast, the selective cytotoxicity of DDE for alveolar macrophages is mediated predominantly by non-cytochrome P450 enzymes. Therefore, although several DDT metabolites are cytotoxic to lung cells, DDD is the most potent cytotoxicant. (Supported by USPHS grant ES05628 from NIEHS)

1479 AGE-DEPENDENT DIFFERENCES IN CARBON TETRACHLORIDE (CCl_4 HEPATOTOXICITY IN FISCHER - 344 RATS

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The potential influence of age on the severity of CCl_4-induced liver injury was examined in young (3 months) and old (22–23 months) male Fischer - 344 rats. All rats received 0, 20, 100, or 500 mg CCl_4/kg in a 10% aqueous emulsion by oral gavage at 3:30pm. Hepatotoxicity was assessed 24H later by measurement of serum alanine - aminotransferase (ALT) and sorbitol dehydrogenase (SDH) activities and by light microscopy. Liver injury was much greater in old animals than in their younger counterparts at each dosage level. For example, after ingestion of 20 mg CCl_4/kg, old animals showed significant elevation of SDH and minimal to mild hepatocellular necrosis. Young animals had no elevation in SDH and no observable necrosis at this dosage. Age-associated changes in the amount of hepatic ALT and SDH available for release into blood were not responsible for the marked differences seen between the age groups. Age-dependent differences in the ability to suppress oxidative damage, as determined by hepatic antioxidant concentrations in control animals, of α-tocopherol and glutathione (GSH), and hepatic activities of GSH-s-transferase, GSH reductase, and GSH peroxidase did not account for the greater toxicity in the old rats. There were no significant differences in hepatic CYP2E1 activity when expressed as nmoles 4-nitrocatechol formed/min/mg microsomal protein. In summary, greater hepatotoxicity was seen in old rats, that could not be explained by differences in levels of cytoplasmic enzymes, hepatic antioxidants or CYP2E1-mediated metabolic activation. (This abstract may not reflect EPA policy. Supported by EPA Cooperative Agreement CR-820447.)

1480 ALTERED SUBCELLULAR DISTRIBUTION OF PROTEIN KINASE C (PKC) ISOZYMES IN C3H10T1/2 MOUSE EMBRYO CELLS EXPOSED TO HALOGENATED AROMATIC HYDROCARBONS (HAH)

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Tumor promoting agents exert their actions through a number of mechanisms to alter gene expression and clonal expansion of carcinogen-initiated cells. 12–0-tetradecanoyl phorbol-13-acetate (TPA) a potent mouse skin tumor promoter is also a potent activator of PKC, a kinase implicated to play a central role in neoplastic transformation, both *in vivo* and *in vitro*. In this study we examined the effects of several HAH type compounds on the activation of various PKC isozymes in C3H10T1/2, a mouse embryo derived cell line. The subcellular distribution of PKC isozymes was determined by Western immunoblotting technique following treatment with polychlorinated biphenyls (PCBs), TCDD or other known tumor promoters. The untreated C3H10T1/2 cells expressed at least three isozymes of PKC namely, alpha, epsilon and zeta with alpha as the predominant isozyme. Treatment with TPA, mezerein or bryostatin (10 ng/ml), agents known to activate PKC, caused translocation of PKC alpha to the membrane fraction indicating the activation of this isozyme. On the other hand, in cells treated for one hr with Aroclor-1254 (a PCB mixture), or 2,4,2',4'-PCB or 2,4,5,2',4',5'-PCB (10 ug/ml) or TCDD (0.1 nM), there was no translocation of the alpha isozyme to the membrane. However, all these agents induced membrane association of PKC epsilon and zeta isozymes under these conditions. Taken together our data suggest that HAH type xenobiotics activate specific PKC isozymes and that this effect may be related to their action as tumor promoters (Supported by a NIEHS Superfund Grant No. P42 ES-04911).

1481 NEUROTOXICITY ASSESSMENT OF AROCLOR MIXTURES ADMINISTERED IN THE DIET TO SPRAGUE-DAWLEY RATS FOR ONE YEAR

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Exposure to polychlorinated biphenyls (PCBs) potentially affects nervous system function. As part of a comparative chronic toxicity/oncogenicity study of different Aroclors (1016, 1242, 1254, and 1260), neurotoxicity was assessed in male and female Sprague-Dawley rats using the functional observational battery and motor activity tests. Doses varied by Aroclor, and ranged from 25 to 200 ppm in the diet. Animals were evaluated prior to dosing and at 13, 26, 39, and 52 weeks of exposure. Data analysis revealed several instances where Aroclor-treated rats were different from control. However, these were considered incidental, lacked any consistent pattern, and did not suggest any definitive Aroclor-induced neurotoxicity. The non-remarkable findings during the four individual assessments were supported by the absence of any treatment-related clinical signs recorded at weekly intervals. While a variety of incidental, spontaneous degenerative changes were found in selected nervous tissue, no lesions were found that could be attributed to neurotoxicity. In summary, 52 weeks of exposure to Aroclors-1016, -1242, -1254, or -1260 mixed in the diet did not yield any functional or morphological changes indicative of PCB-induced neurotoxicity.

1482 TOXICOLOGICAL EVALUATIONS OF THE CFC ALTERNATIVE HFC-236EA

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Toxicological evaluations were performed to assess acute, subchronic and developmental toxicity, cardiac sensitization and genetic toxicity of HFC-236ea. The 4-hr acute lethal concentration of HFC-236ea in rats exceeded 85,000 ppm; narcosis was the predominant clinical sign seen during exposure. Cardiac sensitization in beagle dogs was noted at ≥ 35,000 ppm with a NOEL of 25,000 ppm. No mutagenic/genotoxic activity was found *in vitro* in *S. typhimurium* and *E. coli* mutation assays or in a human lymphocyte chromosome aberration assay at concentrations of <100%. No micronuclei were induced in bone marrow polychromatic erythrocytes in mice exposed at 5000, 25,000 or 50,000 ppm. In 2- and 13-week inhalation studies with rats exposed at 5000, 20,000 and 50,000 ppm (6 hr/day, 5 days/wk), no evidence of body or organ weight effects, clinical pathology, or induction of hepatic peroxisomes were found. Rats exposed to 20,000 or 50,000 ppm displayed a transient, diminished acoustic startle response only during exposure; rats exposed to 5000 ppm were unaffected. Microscopically, dilatation of seminiferous tubules, without effects on germ or Sertoli cells, was observed in rats at 50,000 ppm only in the 13-week study. In a rat developmental toxicity study, maternal toxicity (diminished startle response and weight gain) was evident at 20,000 or 50,000 ppm while no evidence of fetal toxicity was found in any group.

1483 GAVAGE VEHICLE AND VOLUME MODULATION OF BROMODICHLOROMETHANE (BDCM) TOXICITY

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BDCM and chloroform (CHC13) are the most prevalent trihalomethanes resulting from chlorination of drinking water, with BDCM ranking second in concentration only to CHC13. Relative to an aqueous vehicle, corn oil administration of CHC13 or BDCM results in greater hepatic toxicity. When CHC13 is administered in corn oil, a larger gavage volume results in more pronounced nephrotoxicity and hepatotoxicity. The objective of the present study was to examine the effects of gavage volume and vehicle on BDCM-mediated hepatic toxicity. Adult male Fischer-344 rats (~70 days old) were gavaged with 0, 300 or 600 mg BDCM/kg in corn oil or 10% Alkamuls EL-620® solution at a total gavage volume of either 2.5 or 10 ml/kg. Toxicity was assessed at 48 hr by serum markers of hepatotoxicity [alanine and aspartate aminotransferase and sorbitol dehydrogenase (SDH)]. Gavage vehicle and volume had little/no impact on BDCM-mediated decreases in body weight. At 300 mg BDCM/kg, the only indication of hepatotoxicity was increased SDH in the high volume oil group; this was perhaps due to the time of toxicity assessment as other investigators have seen greater elevation of serum indicators at 24 hr than at 48 hr when BDCM was administered in an aqueous vehicle. At 600 mg BDCM/kg, BDCM toxicity was modulated greatly by gavage vehicle with hepatic toxicity greater in the oil vehicle than in the aqueous vehicle. Oil volume effects were prominent, whereas there was little apparent effect due to the volume of the aqueous vehicle. In conclusion, both gavage vehicle and gavage volume affect BDCM toxicity, with administration in the high volume oil vehicle producing the greatest hepatic damage. As human oral exposure to BDCM is primarily through drinking water, an understanding of the mechanism(s) underlying vehicle

and volume differences in BDCM toxicity is needed for interpretation of results from toxicology studies conducted with an oil vehicle. (This abstract does not necessarily reflect EPA policy.)

1484 THE RELATIONSHIP OF SERUM TRIGLYCERIDE (TG), CHOLESTEROL (C) AND GRAVIMETRIC LIPIDS (GL) FOR NORMALIZING PCB AND PESTICIDE LEVELS IN HUMAN SERUM

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Lipophilic xenobiotics such as PCBs and DDE are currently being measured in human serum in a variety of epidemiological studies assessing effects of Great Lakes fish consumption on health outcomes in consumers and their offspring. These and other lipophilic xenobiolics are associated with the lipid component of serum and their concentration in serum has traditionally been expressed as a function of GL content of the serum sample. This approach requires a considerable volume of serum to accurately assess the lipid content and often limits the amounts of serum available for congener specific PCB analysis. In other cases, use of banked samples having limited sample volumes preclude the analysis of GL which requires at least 1 ml of serum. The objective of this study was to compare the relationship of serum GL to serum TG and C in serum samples from 153 individuals taken as part of a study of Great Lakes fish consumption. Linear regression analysis indicated a poor correlation between C and GL (r^2 = .046); a significant correlation between TG against GL (r^2 = 0.441) and the best correlation between (TG plus C) and GL (r^2 = 0.639). Considerable variability was seen in determining GL in samples containing less than 0.5% lipid. Our results indicate that (TG plus C), which can be assayed in 10 to 20 ul of serum, is proportionally related to gravimetric lipid values and may be used as a surrogate measure to correct serum xenobiotic concentrations.

This work was funded in part by a grant from the Great Lakes Protection fund.

1485 EFFECTS OF POLYCHLORINATED BIPHENYLS ON ESTROGEN METABOLISM IN HUMAN BREAST CANCER CELLS

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Polychlorinated biphenyls (PCBs) elicit a broad spectrum of toxic effects. The complexity of PCB toxicity is in part due to the fact that individual congeners elicit markedly different physiologic responses. For example, individual PCB congeners can cause either estrogenic or antiestrogenic effects. Antiestrogenic effects are thought to result from induction of cytochromes P450 (CYP) that catalyze the metabolism of 17β-estradiol (E_2) in estrogen-responsive tissues. In this study two human breast cancer cell lines were used to investigate the effects of PCB congeners on E_2 metabolism; MCF-7, in which both CYP1A1, an E_2 2-hydroxylase, and CYP1B1, an E_2 4-hydroxylase, are induced in response to aryl hydrocarbon receptor (AhR) activation, and MDA-MB-231, in which only CYP1B1 is induced by AhR agonists. Concentrations of the methoxyestrogens, 2-MeOE^2 and 4-MeOE^2, released into the medium were determined as measures of induction of the E^2 2- and 4-hydroxylases. The results showed that none of the non-coplanar congeners tested, including 2,3',4,4',5-PeCB and 2,2',4,4'5,5'-HxCB, affected E^2 metabolism in either cell line. Of the coplanar PCBs, only 3,3',4,4',5-PeCB caused highly elevated production of 2- and 4-MeOE^2 in the two cell lines. Exposure to 3,3',4,4',5,5'-HxCB induced 2-MeOE^2 production two-fold in MCF-7 cells, but inhibited the production of 4-MeOE^2 to below control levels in both cell lines, suggesting inhibition of CYP1B1. These results demonstrate the diverse effects individual PCB congeners have on estrogen metabolism, and thus estrogenic function, in human-derived breast cells. Supported by grant 2P42-ES04913.

1486 REEVALUATION OF ADRENORECEPTOR MEDIATED HEPATOPROTECTION FROM BROMOBENZENE

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Previous studies in mice on protection from bromobenzene-induced heptatotoxicity by phentolamine and idazoxan have been interpreted to imply the involvement of alpha-adrenoreceptors, most likely α2 receptors. Phentolamine is a mixed alpha antagonist and idazoxan was considered α2-selective. Recently, cross-specificity of idazoxan for a different group of receptors, the imidazoline receptors, has been reported, calling into question whether α2 antagonism is responsible for mediating hepatoprotection. A series of other adrenergic antagonists with varying selectivities were tested for hepatoprotective effects on bromo-benzene toxicity. Serum ALT activity, histopathology, and GSH levels were evaluated to characterize hepatotoxicity. When given as a 15 min. pretreatment, the α2-selective antagonist yohimbine (5 mg/kg i.p.), which does not appear to have imidazoline receptor cross-reactivity, failed to protect against liver damage from bromo-benzene (0.45 ml/kg, i.p.). In contrast, the μ1-selective antagonist prazosin (5 mg/kg, i.p.) provided significant protection ($p<0.05$). Pretreatment with the β-selective antagonist propanolol (20 mg/kg, i.p.), resulted in a nonsignificant decrease in ALT levels compared to bromobenzene alone. These results imply that the adrenergic modulation of bromobenzene hepatotoxicity is likely affected by α1, rather than α2, receptors and that the effects of idazoxan may relate to its activity at imidazoline receptors.

1487 COMPARISON OF ORGANOCHLORINE PESTICIDES AND POLYCHLORINATED BIPHENYL RESIDUES IN HUMAN BREAST ADIPOSE TISSUE AND HUMAN SERUM

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Concern exists about the link between organochlorine pesticides, such as p,p'-DDT [2,2-bis(*p*-chlorophenyl)-1, 1, 1-trichloroethane] and polychlorinated biphenyls (PCBs) with human breast cancer. Extensive literature exists supporting residues of these compounds in both human adipose tissue and human serum. However, adequate separation of the pesticides from the PCBs was not done before a Florisil® column chromatography method was developed by the Analytical Laboratory at Colorado State University. Using the newly developed method, accurate residue levels were established for 9 individual PCB isomers as well as six organochlorine pesticides. Mean levels in human breast adipose tissue (n = 15) of p,p'-DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene] were found at 1179.3 ng/g, p,p'-DDT was found at a mean level of 62.4 ng/g, α-Hexachlorocyclohexane (HCH) was not found; β-HCH had a mean level of 30.9 ng/g. Further, PCB mean levels in human breast adipose tissue (n = 15) range from 83.8 ng/g for BZ #180 and 81.1 ng/g for BZ #153 to 14.5 ng/g for BZ# 183 and 23.4 ng/g for BZ #187. Both serum and breast adipose tissue from 25 women will be analyzed and a correlation between serum levels and breast adipose levels will be confirmed. Further, 17-β-Estradiol levels in the serum will also be analyzed. The estrogen levels will then be correlated to both the organochlorine residue levels of the serum and the breast adipose tissue.

1488 NON-COPLANAR POLYCHLORINATED BIPHENYLS (PCBS) INDUCE AP-1 TRANSCRIPTION FACTOR-MEDIATED EXPRESSION OF LUCIFERASE ACTIVITY

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Activating protein-1 (AP-1) is a nuclear transcription factor and induces the transcription of genes that contain AP-1 recognition sequences (TPA response elements or TREs) in their promoter regions. Protein kinase C (PKC) activating agents such as the phorbol ester TPA (12-O-tetradecanoyl phorbol-13-acetate) induce the expression of genes that contain the TREs. Thus, the induction of AP-1 and the ensuing gene expression has been suggested as a mechanism of action of such agents. Since many halogenated aromatic hydrocarbon xenobiotics are epigenetic carcinogens we wished to examine whether exposure to these chemicals induces the expression of heterologous genes that contain TREs. A plasmid containing two TREs in the 5' region of a promoter and a luciferase reporter gene, and a plasmid containing neomycin resistance gene was cotransfected into WB cells, a Fischer F-344 rat liver derived cell line. A clone resulting from this transfection expressed significant luciferase activity in response to treatment with TPA (100 nM). Treatment with Aroclor-1254 (a PCB mixture) or two non-coplanar PCB congeners, 2,4,2',4'-tetrachlorobiphenyl and 2,4,5,2',4',5'-hexachlorobiphenyl (2 ug/ml) for 11 hrs induced a 2- to 4-fold increase in luciferase activity of this clone. The hexachlorobiphenyl congener was more potent than the other agents tested. Our data suggest that the non-coplanar PCBs are potent activators of AP-1 mediated gene activation and that the induction of AP-1

might be related to their action as tumor promoters (Supported by NIEHS Superfund grant No. P42 ES-04911).

1489 TOXICOLOGIC PROFILE OF THE ALTERNATIVE REFRIGERANT, HFC-236FA

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Toxicological evaluations were performed to assess the acute, subchronic, developmental and genetic toxicity, and cardiac sensitization of HFC-236fa, a saturated alternative fluorocarbon refrigerant. The 4-hr acute lethal concentration of HFC-236fa in rats exceeds 195,000 ppm; narcosis was the predominant clinical sign observed during exposure. Cardiac sensitization in beagle dogs was noted at ≥ 150,000 ppm with a NOEL of 100,000 ppm. No evidence of mutagenic/genotoxic activity was found in vitro in Ames or human lymphocyte assays or in vivo in the mouse micronucleus assay. In 2-week and 13-week inhalation studies with rats at 5000, 20,000 and 50,000 ppm (6 hr/d, 5 d/wk), no evidence of body or organ weight effects, clinical pathology, organ pathology, or induction of hepatic peroxisomes were found. Rats exposed to 20,000 or 50,000 ppm had a transiently diminished acoustic startle response only during exposure; rats exposed to 5000 ppm were unaffected. Some adaptation to narcosis was noted as the study progressed. In a rat developmental toxicity study, maternal toxicity (diminished startle response and weight gain) was evident at 20,000 ppm or greater while fetal toxicity was found in any group. The overall no-observed-effect level for HFC-236fa was 5000 ppm, based on narcosis noted at concentrations of 20,000 ppm or greater.

1490 CARBON TETRACHLORIDE (CCL_4) ELIMINATION IN MICE MEASURED BY A SIMPLE BREATH ANALYSIS TECHNIQUE

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Sequential breath samples from CCl_4-treated CD1 mice or deermice (0.1 mmol/kg in 20% Emulphor: saline, i.p.) were measured by gas chromatography(GC). In a biexponential model, elimination half-lives ($t_{1/2}$) were 268.5 and 4.5 min for male CD1 mice. No liver damage, as measured by serum alanine transaminase (ALT) was detected at 24 hrs. At this same dose in deermice, elimination was increased ($t_{1/2}$ = 18.5 and 2.5), and ALT was significantly elevated. It is likely that the increased elimination of CCl_4 was largely due to reductive metabolism of CCl_4 to the toxic metabolite $\cdot CCl_3$. Acetone induction of cytochrome P450IIE1 in deermice increased both elimination and toxicity, while cotreatment with ethanol (4g/kg, p.o.), a competitive inhibitor of cytochrome P450IIE1, decreased both elimination and toxicity. Elimination of a second dose of CCl_4 24 hours after a first dose of CCl_4 (suicide inhibition) was decreased in deermice. GC measurement of the disappearance of CCl_4 from breath is a simple, non-invasive way to measure relative cytochrome P450 activity *in vivo*.

1491 EFFECTS OF PERINATAL AND CONTINUOUS EXPOSURE TO 3,3',4,4'-TETRACHLOROBIPHENYL ON REPRODUCTIVE PERFORMANCE AND SPERM FERTILIZING ABILITY IN MICE

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The objectives of this study were to investigate the effects of 3,3',4,4'-tetrachlorobiphenyl (TCB) on reproductive performance and sperm fertilizing ability in male mice. C57BL/6J female mice (F-0) were treated with 0, 3 ppm, and 30 ppm TCB two weeks before breeding. Each female was then paired with a non-treated male for 10 days. Treatments continued through mating, gestation, and lactation. Male offspring (F-1) were given the same diet as their dams throughout this study. F-1 males at 7, 17, and 60 weeks of age were paired with non-treated females. Body weights, litter sizes, sex ratios, and survival indices of offspring sired by F-1 males were recorded. Sperm fertilizing ability *in vitro* was examined with eggs collected from non-treated females when F-1 males were at 9 and 19 weeks of age. Sperm motion analysis was performed with a computer-assisted program hourly after epididymal sperm were collected. Increased testis weights were observed in the 30 ppm-treated weanlings, but not in pubertal and mature mice. Results of sperm motion analysis for F-1 males were the same among all treatment groups, except for decreased sperm motility observed in 9-week-old 30 ppm-treated males 2.5 hours after collection. The percentage of eggs fertilized *in vitro* by sperm from 30 ppm-treated F-1 males at 19 weeks of age decreased, but not at 9 weeks of age. Increased mortality was observed in offspring sired by 3 ppm- and 30 ppm-treated F-1 males only at 60 weeks of age, but not at 7 and 17 weeks of age. Increased body weights were observed in offspring of 30 ppm-treated F-1 males at 17 weeks of age, but not at 7 and 60 weeks of age. Reproductive performance and sperm fertilizing ability *in vitro* were impaired by TCB exposure. [Supported by NIEHS P42-ESO4911-05]

1492 CCl_4 HEPATOTOXICITY: NOVEL EFFECTS OF VERY LOW LEVELS OF CC14

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The cytoprotective effect of low doses of carbon tetrachloride (CCl_4) has been attributed to activation of cells in GO and S phases to move onto G2 phase and complete cell division, thereby repairing tissue damage produced by the low dose of the agent. We examined the functional aspect of this phenomenon in terms of lipoprotein alteration by administering low doses of CCl_4 to rats via Alzet osmotic minipumps implanted i.p. Our working hypothesis is that as a consequence of tissue repair response, low doses of CCl_4 will stimulate production and release of lipoproteins from the liver. We also measured histochemically lipid retention in the liver, and the role of Ca^{2+} transport in the CCl_4 effect. The lowest dose of CCl_4 (0.07 μl/hr) increased lipoprotein cholesterol (C) by 33%, and at a higher dose (0.35 μl/hr), there was a 16% decrease. Verapamil (3.5 μg/hr) reversed the low dose CCl_4-induced increase of lipoprotein C, but did not affect the higher CCl_4 dose. The decreased plasma lipoprotein level correlated with higher intensity of lipid stains, whereas the increased lipoprotein level correlated with absence or reduced lipid stains, as well as increased cell population in the liver histograms. It is concluded that changes in lipoprotein profile may serve as a functional correlate of the tissue repair response induced by low doses of CCl_4. (Supported in part by Grant #s NHLBIOK14-HL02134, RCMI 1-G12-RR-05075, and ATSDR-AMHPS Fdn.)

1493 GLUTATHIONE S-TRANSFERASE-MEDIATED MUTAGENICITY OF TRIHALOMETHANES

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Trihalomethanes (THMs) are the most prevalent disinfection by-products generated in chlorinated drinking water. Among the THMs, chloroform ($CHCl_3$) generally occurs at the highest concentration in finished water, but the concentrations of the brominated THMs ($CHBrCl_2$, $CHBr_2Cl$, and $CHBr_3$) can exceed that of $CHCl_3$ when source waters with high bromide levels are chlorinated. Each of these four THMs was carcinogenic in rodents in chronic oral dosing studies. The determination of whether carcinogens produce cancer through a genotoxic mode of action is critical in the selection of appropriate risk assessment strategies. The objective of this study was to assess THM mutagenicity in a strain of *S. typhimurium* TA1535 which was transfected with rat GSH *S*-transferase 5–5 (+GST) (Thier *et al., PNAS* 90:8576). The +GST strain and its nontransfected counterpart strain (-GST) were employed in a plate incorporation assay and exposed for 24 hr to the vapor of individual THMs at concentrations from 0-4800 ppm in sealed Tedlar® bags. Base pair revertants were produced in the +GST strain in a dose-dependent fashion by $CHBrCl_2$ and $CHBr_3$, but not by $CHCl_3$. At 3200 ppm, revertants per plate induced by $CHBrCl_2$ and $CHBr_3$ averaged 373 ± 86 and 1935 ± 255, respectively, compared to a spontaneous rate of 23 ± 5. Significant increases in revertants in the -GST strain (44%) and a second TA1535 control strain (124%) were also produced by $CHBrCl_2$, but not $CHCl_3$, at 4800 ppm. These results indicate that bromination of THMs confers the capability for GST-mediated transformation to mutagenic intermediates, and that the *in vivo* genotoxic potential of the BrTHMs may have been previously underestimated. This is the first report demonstrating that glutathione conjugation is an activation pathway for THMs. (This abstract does not necessarily reflect EPA policy.)

1494 CHLOROFORM ADMINISTERED IN THE DRINKING WATER PREVENTS THE TOXICITY AND CELL PROLIFERATION INDUCED IN THE LIVER OF MICE WHEN IT IS ADMINIS-TERED BY GAVAGE IN CORN OIL

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Chloroform (CHCl3) administered by gavage in corn oil but not when administrated in drinking water, has been shown to cause liver cancer in B6C3F1 mice. In fact previous studies of CHCl3 administered in drinking water suggested prevention of liver and colon cancer. Since humans are exposed to CHCl3 in chlorine disinfected water, we evaluated the interaction between CHCl3 administered in drinking water and by gavage in corn oil. The five treatment groups were: (1) CHCl3 (263 mg/kg) administered for three consecutive days by gavage in corn oil, (2 and 3) 300 or 1800 ppm CHCl3 in drinking water for 33 days with 263 mg/kg administered by gavage on each of the last three days, (4) 1800 ppm CHCl3, and (5) vehicle control. Three days before euthanasia, a pellet containing bromodeoxyuridine (BrDU) was implanted subcutaneously into each mouse. Group 1 exhibited extensive hepatic toxicity and proliferation of hepatocytes. The BrDU-labeling index (LI) was 37.2% compared to 3.0% in the vehicle control. Groups receiving 300 or 1800 ppm CHCl3 in drinking water demonstrated a dose dependent reduction in the toxicity and cell proliferation (BrDU-LI = 5.9 and 1.1%, respectively) induced by CHCl3 administered by gavage. The results suggest that CHCl3 administered in the drinking water can prevent the toxicity and likely the carcinogenic activity of CHCl3 administered by gavage in corn oil.

1495 EXPRESSION OF PROTOONCOGENES, TGF-α AND β IN THE LIVER OF B6C3F1 MICE TREATED WITH TRICHLOROETHYLENE, DICHLOROACETIC AND TRICHLOROACETIC ACID

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Trichloroethylene(TCE) is used as a solvent and metal degreaser resulting in measurable quantities in surface water, ground water, ambient air and soil. Its metabolites dichloroacetic acid (DCA) and trichloroacetic acid (TCA), are found as by-products of chlorine disinfection. Exposure of humans to TCE, DCA and TCA is of concern because of their carcinogenic potential. In the present study, we examined the expression of *c-fos, c-jun* and *c-myc* protooncogenes and TGF-α and β, to determine whether they are deregulated in the process of TCE, DCA and TCA-induced mouse liver tumors. B6C3F1 female mice were treated with 800 mg/kg TCE or corn oil vehicle via gavage, once daily for 5 days/week, or with 3.2 gm/L of DCA or 4.0 gm/L of TCA in drinking water. The animals were sacrificed after 1 or 300 days of treatment and for TCE-treated mice at 45, 90 and 120 min after the last dose. mRNAs of the protooncogenes, and TGF-α and β in the liver were analyzed by Northern blottings, and their proteins detected by immunohistochemistry. After a single or 300 days exposure to TCE, *c-jun* expression increased starting at 45 min, peaked at 90 min, then decreased by 120 min; and *c-myc* expression also increased as early as 45 min, but peaked at 120 min. *c-Fos* expression was not detected. TGF-α expression was strongly positive in DCA-induced tumors and negative in non-involved hepatocytes, while TGF-β was strongly positive in the non-involved hepatocytes and negative in the tumors. In TCA-treated mice, the expression of TGF-α and β was much lower and heterogeneous in the non-involved hepatocytes and tumors. The present results suggest that *c-jun, c-myc*, TGF-α and TGF-β may play an important role in TCE, DCA and TCA-induced hepatocarcinogenesis. (Supported by US Air Force Grant No. FQ8671-9401249).

1496 ALKYL POLYCHLORINATED DIBENZOFURANS AS ANTIESTROGENS IN HUMAN BREAST CANCER CELL LINES

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6-Methyl-1,3,8-trichlorodibenzofuran (triCDF) is a relatively non-toxic aryl hydrocarbon (Ah) receptor agonist which exhibits antiestrogenic activity in the female rat uterus and human breast cancer cell lines in culture. *In vivo* studies in the female rat uterus showed that among various structural classes of alkyl polyCDFs, the 1,3,6,8- and 2, 4,6,8-substituted compounds exhibited the highest antiestrogenic/toxic potency ratios. The *in vitro* effects of 8 alkyl polyCDFs were further investigated in both estrogen receptor (ER) negative and positive Ah-responsive human breast cancer cell lines. In ER⁻ MDA-MB-468 cells, 10 nM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induced ethoxyresorufin *O*-deethylase (EROD) activity (45 ± 8 pmol/min/mg) and caused a 53% inhibition of cell growth over a period of 6 days. Five μM concentrations of 6-isopropyl-1, 3, 8-triCDF and 3-isopropyl-6-methyl-1, 8-diCDF induced EROD activity (5 ± 3 and 16 ± 2.7 pmol/min/mg) and the remaining compounds were inactive. In contrast, with the exception of 6-*t*-butyl-1, 3, 8-triCDF, 5 μM concentrations of the remaining alkyl PCDFs caused a 19 to 53% inhibition of cell growth and 6-methyl-2, 4, 8-triCDF exhibited the highest antiproliferative activity. The comparative antiproliferative effects of these compounds in ER⁺ human breast cancer cells were less dramatic and structure-dependent; moreover, higher induction of EROD activity was also observed. The antiestrogenic and induction (EROD) activities of the individual alkyl polyCDFs were thus dependent on the cell line.

1497 CARDIAC SENSITIZATION THRESHOLDS OF HALON REPLACEMENT CHEMICALS PREDICTED BY PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING

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Human exposure to Halon and Halon replacement chemicals is often regulated on the basis of cardiac sensitization potential. The test results are evaluated in terms of a dose-response curve from which No Observed Adverse Effect Level (NOAEL) and Lowest Observed Adverse Effect Level (LOAEL) values are determined. This approach alone does not provide the information necessary to evaluate the cardiac sensitization potential for the chemical of interest under a variety of exposure concentrations and durations. In order to provide a tool for decision makers and regulators tasked with setting exposure guidelines for Halon replacement chemicals, a quantitative approach was established which allowed exposures to be assessed in terms of the chemical concentrations in blood during the exposure. A Physiologically Based Pharmacokinetic (PBPK) model was used to simulate blood concentrations of Halon 1301, HFC-125 (pentafluoroethane, CHF_2CF_3), HFC-227ea (heptafluoropropane, CF_3CHFCF_3, HCFC-123 (dichlorotrifluoroethane, $CHCl_2CF_3$) and CF_3I (trifluoroiodomethane) during inhalation exposures. This work demonstrates a quantitative approach for use in linking chemical inhalation exposures to the levels of chemical achieved during the exposure. (Supported by Dept. of the Air Force Contract No. F33615-90-C-0532)

1498 PRODUCTION OF CARBON MONOXIDE AND CARBOXYHEMOGLOBIN DURING DESFLURANE, ENFLURANE, ISOFLURANE, AND SEVOFLURANE ANESTHESIA IN DOMESTIC SWINE

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In some circumstances carbon monoxide (CO) maybe produced in the CO_2 absorbent during anesthesia with halogenated anesthetics. The production of CO has been linked to the dryness (i.e. water content) of the CO_2 absorbent. In our study, after drying the CO_2 absorbent (Baralyme) for 24 h we compared arterial carboxyhemoglobin (COHb) and CO levels in the anesthesia circuit during 4 h of desflurane (D), enflurane (E), isoflurane (I), and sevoflurane (S) anesthesia in domestic swine (25–30 kg). Baralyme was dried by flushing a canister set for 24 h with 10 l/min of 100% O_2. Pre-and 24 h samples of Baralyme were collected to determine water content. Femoral artery was exposed and cannulated with a 20 g angiocatheter. Samples for COHb were taken at pre, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150, 180, 210, and 240 min. COHb samples were analyzed with a co-oximeter to measure %COHb. CO samples were collected from the vaporizer circuit in 5 ml gas tight syringes at pre, 5, 10, 15, 20, 30, 60, 90, 120, 150, 180, 210, and 240 min. CO samples were analyzed by injecting 5 ml of sample into a gas chromatography with a thermal conductivity detector and a molecular sieve column (60/80 mesh). Signs of CO poisoning (deep red skin color, irregular heart beat, and reduction in expired CO_2) occurred at 20–40 min during D anesthesia. The drying of the Baralyme water content to 10% or less produced elevated COHb levels of 72.96 ± 2.45 % with D, 58.58 ± 7.86% with E, and 21.40 ± 0.66 with I anesthesia. Sevoflurane did not produce elevated COHb or CO. Drying of Baralyme to a degree capable of producing dangerous levels of CO can develop in the clinical situation. The drying of Baralyme can result in dangerously high CO and COHb levels during D, E, and I anesthesia.

1499 DIMETHYL SULFOXIDE HAS A LATE HEPATO-PROTECTIVE EFFECT AGAINST CHLOROFORM AND BROMOBENZENE INDUCED LIVER INJURY IN THE RAT

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Dimethyl sulfoxide (DMSO) has previously been shown to help protect against the development of hepatic necrosis in the guinea pig when administered 10 hr after inhalation exposure to halothane. To further explore the range of hepatoprotection provided by DMSO, male Sprague-Dawley rats (300–325 g) were administered either 1.0 mg/kg chloroform (C) or 0.5 ml/kg bromobenzene (B), 20% in corn oil, po. At 10 hr after toxicant administration, animals received either 4 ml/kg saline (S) or 2 ml/kg DMSO, 50% in saline, ip. DMSO had hepatoprotective properties against both toxicants. Plasma ALT activity at 48 hr after toxicant administration was decreased by DMSO treatment (C + S = 5,230 ± 602 U/L, C + DMSO = 493 ± 191, B + S = 7,940 ± 720, B + DMSO = 1,670 ± 1,420, $p < 0.01$, x ± SEM, control = 35 ± 2, N=5–6). The extent of resultant zone 3 necrosis was also markedly reduced by DMSO in both experiments. These findings provide further evidence for the ability of DMSO to interrupt the progression of xenobiotic-induced hepatic necrosis. The late time of DMSO administration would indicate that the observed beneficial effects are due to a mechanism other than inhibition of P-450 based biotransformation. (P30-ES-06694).

1500 EFFECT OF PRE-EXISTING ANTI-TFA ANTIBODIES ON THE HUMORAL IMMUNE RESPONSE AND HEPATOTOXICITY AFTER SUBSEQUENT HALOTHANE EXPOSURE

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Drug induced hypersensitivity response after halothane administration is presently considered the cause to halothane hepatitis (HH) since HH patients have antibodies specific for the trifluoroacetyl (TFA) protein adduct of halothane and HH almost always occurs after multi-exposures. This study has focused on investigating if pre-existing anti-TFA antibodies would potentiate the humoral immune response and hepatotoxicity with subsequent halothane exposures. Nine male Hartley guinea pigs were immunized with synthetic trifluoroacetyl conjugated guinea pig serum albumin (TFA-GSA) twice at 20-day intervals. Twenty days after the last TFA-GSA immunization, guinea pigs were exposed to 1% halothane for 4 hr. The guinea pigs were examined for anti-TFA antibodies, serum liver enzymes, liver histopathology and immunohistochemistry for 6 wk after the halothane exposure. Anti-TFA IgG titers were higher (max at 3 wk after halothane) in the guinea pigs immunized with TFA-GSA compared to age related guinea pigs who only received halothane. However, the serum ALT and immunoperoxidase staining for detection of halothane adducts in liver section were not different between the two groups. These data suggested that although the subsequent challenge of immunized animals with 1% halothane generated a secondary antibody response which was significantly greater than the nonpretreated animals, the sensitization did not increase halothane hepatotoxicity.

1501 MORPHOLOGICAL CHANGES IN TISSUE FROM MRL +/+ MICE DUE TO TRICHLOROETHENE AND DICHLOROACETYL CHLORIDE TREATMENT

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Trichloroethene (TCE) is widely used in industry as a degreasing agent and is a common environmental contaminant. A previous study in our laboratory has shown that treatment with TCE or dichloroacetyl chloride (DCAC, a metabolite of TCE) causes the production of various autoantibodies in female MRL +/+ mice after 6 weeks. The present study describes a morphological evaluation of tissues obtained from TCE or DCAC-treated mice. Both a dose response (0.05, 0.1, 0.2, 0.4 mmole/kg, every 4th day for 6 weeks) and a time response (0.2 mmole/kg every 4th day for 2, 4, 6 or 8 weeks) study were performed for DCAC, while TCE was only examined at a dose of 10 mmole/kg every 4th day for 6 weeks. The primary lesion in all treatment groups was in the spleen, where a loss of cellularity in the white pulp and a gain in cellularity of the red pulp was observed, along with a large accumulation of neutrophils and macrophages. The white pulp loss was seen in the demarcation between the marginal and central zones. These cellularity changes were observed in a dose-response relationship in DCAC-treated animals, however, a time-response relationship could not be established. Besides the spleen, other organs showed major morphological changes. In the lung, the primary lesion was a mild alveolitis characterized by a patchy thickening of the alveolar septa, leukocyte infiltration, and enlargement of the air spaces. Liver lesions increased in a dose-dependent manner, and included increased numbers of mitotic figures, small foci of inflammatory cells, and areas of hemorrhage. We conclude that similar morphological changes are produced in female MRL +/+ mice given either DCAC or TCE, and that these changes occur in a dose-dependent manner rather than a time-dependent manner. (SMG is supported by T32ESO7254).

1502 GENETIC DETERMINANTS OF STABLE LEAD RESISTANCE IN RAT GLIOMA CELLS

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To isolate genes which are overexpressed in lead-resistant cells, a number of rat glial tumor cell (C6) sublines that are lead-resistant were isolated. Isolation of lead-resistant sublines was accomplished by increasing the concentration of lead nitrate in the medium every two weeks up to a concentration of 300 μM. Cells growing in 300 μM lead nitrate were re-seeded in medium containing concentrations of lead nitrate up to 550 μM. Several lead-resistant colonies surviving in the highest concentration of lead nitrate were picked and grown in the absence of lead for long periods of time. After more than eight months of growth, three varient sublines maintained their lead-resistant phenotype. In order to fully characterize the phenotypes of these variants, their sensitivities to other metal salts were also determined. Cytotoxicity was determined by clonal assay. Lead-resistant variants are not cross-resistant to Cd(II) or Ni(II), but they are all cross-resistant to Hg(II), As(III), Sb(III) and Sn(II). We have started to clone cDNA's which confer lead resistance. To identify and analyze altered gene expression at the mRNA level in lead-resistant variants, the differential display technique was used. Re-amplified cDNA fragments are now being cloned into appropriate vectors for sequencing. Supported by EPA Grant #R82- 0984.

1503 GLUTAMATE UPTAKE AND GLUTATHIONE CONTENT IN LEAD-TREATED ASTROGLIA

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Low-level lead (Pb) exposure has been implicated in cognitive, attentional and behavioral deficits in children. Current research is aimed at identifying cellular markers of toxicity in order to explain these deficits. Because astroglia have been shown to accumulate Pb and are no longer considered passive cells in some neurological toxicities/disorders, this study examined two parameters of astroglial metabolism: glutamate uptake and glutathione (GSH) content. Astroglia were treated with 1 μM Pb for 1, 3, 7, 14 and 21 days. Preliminary data revealed that uptake of [^{3}H]-glutamate was reduced with Pb exposure. Intracellular GSH levels initially decreased, but increased to beyond control levels with continuous Pb exposure. Because of the importance of GSH in essential cellular processes and in cellular membrane integrity, our findings of altered GSH levels and glutamate uptake may be related and may have important implications in the pathogenesis of Pb neurotoxicity. Funded by NIH grant #K11-ES99279-01

1504 NEURONAL NITRIC OXIDE SYNTHASE ACTIVITY IN RATS EXPOSED TO LEAD IN DRINKING WATER

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Lead (Pb) is a known modulator of calcium signaling pathways. Many of the neurotoxic effects of lead appear to be due to the ability of lead to mimic or alter Ca^{2+} mediated cellular processes. Therefore we studied the *in vivo* effect of Pb exposure on nitric oxide synthase (NOS), a recently discovered calcium dependent enzyme which mediates the formation of nitric oxide. Adult male Spraque-Dawley rats were divided into different groups and were exposed to lead (0, 100, 250 and 500ppm lead acetate in drinking water) for 15 days. In the other two groups one group received NOS inhibitor L-nitro arginine methyl ester (L-NAME) (10mg/Kg, IP/daily) and the second group in addition to L-NAME received 500ppm of lead acetate in drinking water. At the end of two weeks the rats were sacrificed, brains were removed,

fractionated and NOS activity was determined. The rats exposed to Pb showed a decrease in brain NOS activity but was not significant as compared to controls. The group receiving L-NAME + 500ppm Pb acetate also showed a decrease in brain NOS activity but was not significant compared to the L-NAME treated rats. However, L-NAME treatment alone significantly inhibited NOS activity. These results suggest, in the present study, lead toxicity in brain is not via the L-Arginine-NO pathway at the dose and duration of exposure.

1505 ATTENUATION OF DOPAMINERGIC ACTIVITY IN NUCLEUS ACCUMBENS OF RATS EXPOSED TO LEAD

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Long-Evans hooded (21-day-old, male) rats were exposed to either sodium acetate (control) or 50 ppm lead acetate in deionized distilled water for 90 days and tissue contents of dopamine (DA) were studied in nucleus accumbens (NA). Pb measurements were carried out using atomic absorption spectrophotometry equipped with graphite furnace while DA was measured with HPLC coupled with electrochemical detection. The exposure protocol produced blood Pb levels of 18 ± 2 μg/dl, brain Pb levels of 350 ± 20 ng/g wet tissue; and resulted in significant reduction in DA contents (50%) in NA. These studies were extended to examine whether the reductions in DA contents were associated with alterations in basal and stimulus (100 mM K^+) induced release of DA in this brain region employing the microdialysis technique. After the 90-day exposure period, rats were anesthetized with urethane and 2 mm microdialysis probes were implanted in NA (AP 2.7 mm, L 1.5 mm, V −7.5 mm) using a stereotaxic apparatus and perfused with artificial CSF (aCSF) at a rate of 2 μl/min. The perfusates were analyzed for DA. Consistent with the observations of reductions in DA contents, the microdialysis studies also indicated significant reductions in the basal and potassium induced release of DA in Pb treated rats. These results indicate that subchronic exposure to Pb results in reduced dopaminergic activity in NA. Supported by ATSDR/MHPF Grant No. U50/ATU398948-03.

1506 EFFECT OF CHRONIC EXPOSURE OF LEAD ON INOSITOL POLYPHOSPHATE RECEPTOR BINDING IN RAT BRAIN

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We have previously demonstrated *in vitro* that lead alters neuronal calcium homeostasis by the modulation of inositol 1,4,5-trisphosphate (IP_3) and inositol 1,3,4,5-tetrakisphosphate (IP_4) receptors. In the present investigation, IP_3 and IP_4 receptor binding activities and calcium-mediated catabolic enzymes such as calpain and endonuclease were studied in cerebellum and cerebral cortex of rats chronically treated with lead. Adult male Sprague-Dawley rats were exposed to 500 ppm of lead acetate through drinking water for a period of 8 weeks (wk) and they were sacrificed at intervals of 1, 4 and 8 wk. Brains were quickly removed and quick frozen in liquid nitrogen. ^{3}H-IP_3 and ^{3}H-IP_4 were used to determine the receptor binding. Calpain was determined by using alkali-treated casein and endonuclease was estimated by determining the DNA content. ^{3}H-IP_3 and ^{3}H-IP_4 receptor binding activities were significantly increased both in treated cerebellum and cerebral cortex but the increase was more in cerebellum. The kinetic analysis of ^{3}H-IP_3 receptor binding revealed that the K_D values were decreased significantly in treated cerebellum without change in B_{max}. The kinetic constants were not altered in treated cerebral cortex. There was an increase in endonuclease and calpain activities in treated cerebellum at 1wk. However, the increase in endonuclease activity was continued at 4 and 8 wk but the calpain activity decreased at 4 and 8 wk in treated cerebellum. These enzyme activities were not significantly altered in treated cerebral cortex. The above data support our earlier *in vitro* studies that lead alters IP_3 and IP_4 receptor binding activities in brain. The increase of these receptor activities by lead increases the cytosolic calcium which is detrimental to neuronal function. (Supported by NIH/NIGMS/MBRS 08169).

1507 EFFECTS OF LEAD ON [^{3}H]MK-801 BINDING TO NMDA RECEPTOR IN THE BRAIN OF RAT

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Lead is known to be a potent neurotoxin which causes neurological damage and behavioral disruptions in experimental animals. Several lines of evidence suggest that N-methyl-D-aspartate (NMDA) receptor is directly involved in learning and memory functions, and protein kinase C (PKC)-dependent phosphorylation may be important for the function of NMDA receptor by controlling the threshold level for the induction of long term potentiation. We investigated effects of lead on PKC and NMDA receptor binding activity of the rat brain by autoradiographic methods using [^{3}H]phorbol 12,13-dibutyrate (PDBu) and [^{3}H]MK-801 as ligands. Adult male rats were administered lead acetate 100 mg/kg or sodium acetate 36 mg/kg (control) by i.p. for 7 days. The brains were sectioned and used for [^{3}H]PDBu and [^{3}H]MK-801 binding assay. The distribution of PKC and NMDA receptor in twenty-eight areas of the brain were examined. Results indicated that the distribution of [^{3}H]PDBu and [^{3}H]MK-801 binding is very similar with the following order of binding densities: hippocampus>cerebral cortex>caudate-putamen>thalamus>brain stem. High density of PKC and NMDA receptor found in hippocampus is consistent with the hypothesis that the hippocampus may be a critical neural structure for learning and memory processes. No significant effects of lead on PKC and NMDA receptor was found in most regions. However, lead produced significant decrease in [^{3}H]MK-801 binding in the hippocampus (fields CA 1–3 of Ammon's horn) and frontal cortical layers ranging from 10 to 22%. Lead also inhibited [^{3}H]PDBu binding in dentate gyrus and presubiculum by 15% ($P<0.01$) and 20% ($P<0.05$), respectively. Our results support the ideal that the hippocampus appears to be a primary target for lead toxicity. PKC and NMDA receptor alterations in the hippocampus may mediate some of the behavioral changes observed in lead-exposed animals.

1508 ALTERATIONS IN THE METABOLIC HEAT RATE OF PC12 CELLS AFTER EXPOSURE TO LEAD ACETATE COULD INDICATE INTERACTION BETWEEN Pb^{2+} AND Ca^{2+}

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The interaction between lead and essential metals is a complex, well recognized, but poorly understood phenomenon. Lead may alter the homeostasis and function of essential metals. This study focuses on the effect of lead on calcium(Ca^{2+}). Calcium has elaborate systemic and cellular homeostatic, and innumerable second messenger and coupling-factor functions. We have measured the effect of Pb^{2+} (0 to 25μg/dL range) on the metabolic heat rate (MHR) in a model cell line, PC12 cells, using a differential scanning calorimeter in the isothermal mode. MHRs of PC12 cells increased with increasing concentrations of lead acetate. This observed increase in metabolic heat may have several explanations. Lead may possibly perturb calcium-regulated or calcium-mediated functions in the cells (a) by interfering with calcium transport or storage processes (b) by altering cell functions required for calcium homeostasis and/or (c) by substituting Pb^{2+} for Ca^{2+} at functionally important Ca^{2+} binding sites, eg. calmodulin. We have chosen to study the possibility that lead may be mimicking or substituting for calcium at functionally important Ca^{2+} binding sites. To test this latter explanation, MHR of PC12 cells with added concentrations of $CaCl_2$ (in the presence and the absence of A23187 ionophore) were measured. In addition, the enzymatic activity of a metabolic enzyme, glucose-6-phosphate dehydrogenase was measured in the lysates of lead-exposed PC12 cells to demonstrate that the observed increased MHR may be due in part to acceleration of one or more calcium-dependent enzyme-catalyzed metabolic reactions.

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1509 CHRONIC EXPOSURE TO INORGANIC LEAD MODIFIES THE DEVELOPMENT OF ALPHA (α), BETAII (βII), AND GAMMA (γ) PROTEIN KINASE C (PKC) ISOZYMES IN THE RAT HIPPOCAMPUS

M T Madden, V Miletic. *Environmental Toxicology Center, University of Wisconsin-Madison, Madison, WI.* Sponsor: A Elfarra

The aim of this study was to examine the effect of chronic inorganic lead exposure on the development of the subcellular distribution of PKC γ, βII, and α isozymes in the rat hippocampus. Dams were exposed to either 0 or 1000 ppm lead acetate in their drinking water and mated. Offspring were exposed in utero, via lactation, and directly in the drinking water after weaning. The offspring were sacrificed at postnatal days 1 (P1), 8 (P8), 15 (P15), and 29 (P29). Blood lead levels were determin d at these postnatal periods and correlated to the amount of lead given. The amount of each isozyme was

determined in the hippocampal cytosolic and crude P-2 membrane fractions by semi-quantitative and quantitative immunoblotting techniques. Chronic lead exposure differentially affected the development of the hippocampal α, βII, and γ PKC isozymes. The γ isozyme was affected at all measured postnatal periods. Lead decreased the amount of the γ isozyme in the cytosolic fraction at P1 in both fractions at P15, and in the membrane fraction at P29. However, lead increased membrane γ PKC at P8. Lead decreased the amount of βII in the membrane fraction at P1, whereas it increased the amount of βII in the membrane fraction at P15. Lead also increased the amount of the α PKC isozyme in both fractions at P15. The results demonstrate that chronic lead exposure differentially modifies the development of the α, γ, and βII PKC isozymes at specific postnatal periods This suggests that lead's action on PKC is mediated by multiple mechanisms which can contribute to lead's neurotoxic action in the developing rat hippocampus. (Supported by NIH NS21278).

1510 IN VIVO AND IN VITRO INTERVENTION OF CHOROID PLEXUS (CP) PROTEIN KINASE C (PKC) BY LEAD (Pb)

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Pb exposure reportedly stimulates PKC activity in the blood-brain barrier. Pb accumulates in the CP, which serves as the blood-CSF barrier. We therefore evaluated PKC activity in the CP of rats exposed to Pb in drinking water (0, 50 or 250 μg Pb/ml) for 30, 60 or 90 days. CP PKC activity was assayed by incorporation of radiolabel from [γ-^{32}P]ATP into lysine-rich histones following a semi-purification procedure. Pb exposure did not significantly alter either cytosolic or membrane-bound PKC activities in the CP at all tested doses and times by two-way ANOVA. For comparison purposes, we developed a primary rat choroidal epithelial cell culture and used this in vitro system to investigate Pb toxicity. The CP culture from 4 week-old rats showed a dominant polygonal type of epithelial cells for at least 7–10 days with a doubling time about 3–4 days. Thus, we used 5-day-old cultures for our study. The cells were incubated with Pb for 50 min, and the cell homogenate assayed for PKC activity. Exposure to Pb (1.0 and 10 μM) resulted in 3.4 and 4.5 fold, respectively, increases in membrane-bound PKC activities, while cytosolic PKC activities were significantly decreased ($p < 0.05$). Our in vitro results were consistent with those reported for the blood-brain barrier, suggesting a possible role of Pb in modulating cellular PKC by translocation of it from the cytosol to membrane. However, the explanation as to why the in vitro and in vivo results are discordant remains to be determined. (Supported in part by Grant P20-ES-06831-01 and the Lucille Markey Foundation.)

1511 LEAD DISTRIBUTION BETWEEN CORTICAL AND TRABECULAR BONE IN AN IMMATURE SWINE MODEL

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Exposure of children to environmental lead remains one of the most significant public health problems in the US. We have employed an immature swine model to estimate site-specific lead absorption in children (Weis et al., 1995). Use of the immature swine and other animal models to predict lead absorption and distribution has improved the cost efficiency of public health protection in residential areas known to be contaminated with lead in soil and dust.

To better characterize the immature swine model and to further our understanding of lead distribution in bone, we have recorded the dose dependence of lead absorption into whole skeleton and into the cortical and trabecular compartments of both tibia and humerus. Groups (N = 5) of intact weanling boars weighing between 10 and 12 kg were randomly segregated, individually housed, and dosed twice daily for 25 days with either soluble lead acetate (0, 25, 75, 225 or 675 ug/kg-d) or lead-contaminated residential soil (0, 90, 225, 560, or 1400 ug/kg-d). An intravenous dose (100 ug/kg-d N = 3)) was included as an absolute absorption reference. Regular blood lead measurements were recorded and will be reported elsewhere. At study termination, lead concentration was determined in liver, kidney, brain, skeletal muscle and the bone compartments indicated above.

Our results quantify linear, dose-dependent distribution into all bone compartments measured. Preferential absorption of lead into trabecular network in both tibia (~0.31 ± 0.3 ppb per ug/kg-d) and humerus (~0.32 ppb per ug/kg-d) is evident for lead acetate. By contrast, the deposition of lead acetate into cortical bone was significantly less for both tibia (~0.20 ppb per ug/kg-d) and humerus (~0.23 ppb per ug/kg-d). Our results clearly demonstrate the preferential deposition of lead into trabecular network of immature swine and allow definition of a valid reporter bone for estimation of whole skeletal lead concentrations in this test system.

1512 PREPARATION AND BIODISTRIBUTION OF PB-203 COMPLEXES DERIVED FROM MESO AND RACEMIC DMSA

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Meso-dimercaptosuccinic acid (*meso*-DMSA) is one of the drugs of choice in the treatment of lead (Pb) intoxication. Knowledge concerning the biodistribution of this compound and its complexes is limited, and it is not known whether any lead complexes derived from *meso*-DMSA are formed *in vivo* (Aposhian, 1995). We hypothesized that radiochemical techniques using Pb-203 could be used to study Pb complexes of *meso*-and rac-DMSA *in vivo*. Radiolabeled complexes were prepared by eluting Pb-203 from chelex ion exchange columns with *meso*- and racemic-DMSA solutions, and were administered to mice by tail vein injection. Animals were sacrificed between 5 min and 6 hrs. Organ activity was expressed as percent injected dose (% ID). The data were analyzed and compared with biodistribution data obtained from intravenous injection of ^{203}Pb-chloride. The radioactivity balance of ^{203}Pb in the course of the study was 95% ID in tissues and carcass. In the case of the complexes, only 50% ID was left at 30 min and subsequent time points. This is evidence that DMSA complexes of lead are rapidly excreted. The time activity curves for blood, intestines, kidneys, and femur were compared. Clearance of activity from the blood and kidneys was rapid, while activity in the intestines remained constant throughout. This suggests that renal clearance is the primary mode of excretion. Bone uptake and accumulation was observed after the administration of $^{203}PbCl_2$. After the 50% clearance of activity originating from the complexes, accumulation of activity in the bone over time was noted. These studies show that half of the radioactivity derived from DMSA-Pb complexes is excreted quickly, presumably in the form of a complex, while the remainder of the radioactivity may be in another chemical form. The results indicate that the preformed DMSA-Pb complexes have limited stability *in vivo*. This investigation sets the stage for future work that may elucidate chemical aspects of Pb chelation with *meso*- and rac-DMSA *in vivo*.

1513 THE PROTECTIVE EFFECT OF DMPS AGAINST MERCURY TOXICITY IN THE ISOLATED-PERFUSED PROXIMAL TUBULE OF THE RABBIT KIDNEY

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When isolated S2 proximal tubular segments were perfused through the lumen with 20μM inorganic mercury (Hg^{++}) and were bathed in 200μM 2,3-dimercapto-1-propanesulfonate (DMPS), the toxic effects of the Hg^{++} were prevented. This was determined by the low leak rate (0.18 ± 0.06 nl $min^{-1}mm^{-1}$) of the volume marker (^{3}H-L-glucose), the normal appearance of the epithelial cells, and the lack of uptake of the vital dye (FD&C green) into the cells. In contrast, tubules perfused with 20μM Hg^{++} only, had higher leak rates (0.6 ± 0.12), while the epithelial cells swelled and blebbed and took up the vital dye within ten minutes of beginning perfusion. In addition, the disappearance flux rate of Hg^{++} from the lumen and the appearance flux rate of Hg^{++} into the bathing solution were both lower when DMPS was present (20.17 ± 3.98 vs 82.30 ± 22.18 and 8.00 ± 2.95 vs 20.37 ± 6.80, fmol $min^{-1}mm^{-1}$, respectively). Also, less Hg^{++} was bound to large cellular proteins when DMPS was present (117.42 ± 67.67 vs 664.70 ± 240.26 fmol mm^{-1}). It appears that DMPS is transported from the bathing solution into the lumen where it binds to Hg^{++}, presumably forming a coordinate covalent complex, which reduces the entry of Hg^{++} into the epithelial cells and prevents cellular damage.

1514 THE EFFECTS OF TWO CHELATING AGENTS, DMPS AND DMSA, IN REDUCING MORTALITY IN EARTHWORMS TREATED WITH $HgCl_2$

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An examination was made in which two time studies were done where earthworms, *Lumbricus terrestris*, were first treated via injection into the

coelom with the determined LD_{50} value of 49 μM for $HgCl_2$. After this treatment, the earthworms then underwent chelation therapy using one of the two chelating dithiols, 2,3-Dimercapto-1-propanesulfonic acid (DMPS) or *meso*-2,3-Dimercaptosuccinic acid (DMSA), in equal molar concentration to the LD_{50} of $HgCl_2$. Chelation therapy was administered 5, 10, 20, 40, and 80 minutes after initial exposure to the $HgCl_2LD_{50}$ value. From our results, the critical period of effectiveness of DMPS was found to be between the 20 and 40 minute time intervals, whereas the critical period of effectiveness of DMSA was found to be between the 40 and 80 minute time intervals. Contrary to current literature, it appears as if DMSA is the more effective dithiol for two reasons: 1.) More worms survived overall after treatment with DMSA and 2.) more worms survived over a longer period of time after initial $HgCl_2$ exposure upon treatment with DMSA than DMPS.

1515 INTERRELATIONSHIP BETWEEN ADRENALS AND TRIMETHYLTIN (TMT) TOXICITY

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Hippocampus is the primary target site of TMT neurotoxicity and is also prime site for corticosterone uptake. The present investigation is aimed to explore the inter-relationship between adrenals and TMT exposure and neurotoxicity. After TMT administration (1–3 mg/kg) to mice, a dose corresponding increase in the thickness of the zona reticularis (eosinophilic X-zone) with a reduction in zona fasciculata was observed. These changes suggest an increase in physiological stress and ACTH production in these animals. Corresponding dose-related neurotoxicity was also evident. An enhancement of hippocampal damage was observed in adrenalectomized animals. On the other hand, such lesion can be significantly reduced with supplementations of corticosterone. Our present study indicates that 1. TMT neurotoxicity may be enhanced by physiological stress and 2. as corticosterone is believed to have an inhibitory effect on the hippocampal neurons, the reduction of TMT-induced lesion by corticosterone further affirms the hypothesis that hyperexcitation of hippocampal neurons constitutes one of the mechanistic bases of TMT neurotoxicity.

1516 TRIMETHYLTIN STIMULATES PROTEIN KINASE C TRANSLOCATION BY ACTIVATION OF PHOSPHOLIPASE C COUPLED RECEPTORS

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Trimethyltin (TMT) is a potent neurotoxic organotin compound which causes memory and learning perturbations due to its ability to cause hippocampal neurodegeneration in the mammalian brain. Recent findings in our laboratory have shown that TMT exposure leads to protein kinase C (PKC) activation which plays a significant role in TMT-mediated cell death in the PC12 cell. Here we report the mechanism of TMT-stimulated PKC translocation results from the activation of phospholipase C (PLC), presumably through the activation of plasma membrane receptors coupled to PLC. TMT-stimulated PKC translocation was blocked by pretreating cells with U73122 (10 gmM), an inhibitor of PLC. TMT-stimulated PKC translocation was also significantly decreased by pretreating cells with a combination of atropine (30 gmM) and (pl)-alpha-methyl-4-carboxyphenylglycine (MCPG, 1 mM), antagonists to the cholinergic muscarinic receptor and glutamatergic metabotropic receptor, respectively. These results are consistent with earlier findings where TMT-stimulated inositol 1,4,5-trisphosphate formation, a coproduct of PLC activation, can be blocked by pretreating with U73122 or copretreatment with atropine and MCPG. This data along with the observation that TMT stimulates translocation of non-calcium dependent PKC isozymes in these cells suggests translocation occurs in response to increased phospholipid second messengers, namely diacylglycerol, a coproduct of phospholipase C activation.

1517 COCAINE INHIBITS MANGANESE ACCUMULATION IN THE VENTRAL MESENCEPHALON OF RATS

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After intrathecal (i.th.) Mn^{2++} (250 μg Mn/rat as $MnCl_2$), Mn levels in the caudate-putamen, ventral mesencephalon, occipital pole, frontal cortex and cerebellum were determined by graphite furnace atomic absorption. Six hours post-intrathecal Mn, the ventral mesencephalon had the largest amount of Mn (59.3 μg Mn/g); the next largest occurred in the occipital pole (12.1 μg Mn/g); dopamine levels in the caudate-putamen determined by HPLC-ECD were 52% of controls. These results suggested that Mn may be transported into the ventral mesencephalon as a dopamine complex via the dopamine re-uptake transport system. To test this, animals were pretreated with the competitive dopamine re-uptake inhibitor, cocaine (8.6 mg/kg i.p., the in vivo ED_{50} dose) or 0.9% saline. Thirty minutes later rats were given either i.th. Mn^{2++} (250 μg Mn/rat) or equal amounts of Cl-. Six hours after the i.th. administration, rats were euthanized and brains collected. In rats receiving i.th. Cl-, cocaine administration did not alter the regional distribution of Mn in the brain. In rats receiving i.th. Mn^{2++}, cocaine administration significantly reduced Mn to 26%, 34% and 46% in the ventral mesencephalon, occipital pole and frontal lobe, respectively, when compared to rats receiving saline (i.p.) + Mn^{2++} (i.th.). Dopamine levels were reduced in all experimental animals when compared to controls. These results support the hypothesis that dopamine transport is involved in the accumulation of Mn in the ventral mesencephalon. (Support: Superfund Program NIEHS #ES-04940 & NIEHS Center Grant #P30-ES-06694.)

1518 METAL-BINDING PROPERTIES OF PHYTOCHELATINS (PCs)

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Glutathione(GSH)-derived PCs with the general structure $(\gamma GluCys)_nGly$ (n = 2–11) detoxify heavy metals in plants and some yeasts. Most predominant PCs in plants and yeasts are $(\gamma Glu\text{-}Cys)_2Gly$, $(\gamma Glu\text{-}Cys)_3Gly$ and $(\gamma Glu\text{-}Cys)_4Gly$. We have undertaken an integrated approach to answer the following important questions: (i) how are metals presented to PCs?, (ii) what is the relationship between the metal-binding stoichiometry and the chain length of a given PC? and (iii) are metals transferred from shorter chain to longer chain PCs during their biosynthesis? The metal-induced charge-transfer transitions in the optical spectra and alterations of retention times on reverse-phase C_{18} columns were used to define the metal-binding characteristics of PCs. Based upon characterization of Cu(I), Ag(I), Hg(II), Pb(II) and Bi(III)-binding to PCs, we conclude that: (i) the metal ions bound to GSH are easily transferred into PCs indicating that this tripeptide is the general *in vivo* donor of metal ions to PCs, (ii) despite their short length, PCs are capable of forming multiple metal-containing species depending on the PC chain length and the metal ion bound, (iii) metal-binding stoichiometries of PCs, in general, increase with their chain length and (iv) the metal ions are transferred from shorter chain to longer chain PCs. These studies suggest a model in which the metal ions are initially sequestered by GSH and then transferred to PCs. As peptide chain elongation occurs, the metal ions are transferred from shorter to longer chain PCs.

1519 CLONING AND CHARACTERIZATION OF COMMON CARP METALLOTHIONEIN CDNAS

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We are using common carp as a tropical fish model to study consitutive metallothionein (MT) gene regulation in fish tissues in relation to metal ion metabolism in fish. A cDNA library of common carp (*Cyprinus carpio*) liver (uninduced) was screened for metallothionein (MT) cDNA clones using a flounder MT cDNA probe. Among the 13 positive clones that we obtained their nucleotide sequences, 11 were found to carry an identical cDNA (clone 3–14) and two other variant MT cDNAs (clones 5–2 and 3–4) that carry different 5'-UTRs. However, these three MT cDNAs have identical open reading frame which encodes a MT of 60 amino acid residues. One of the variant carp MT cDNAs (clone 5–2) which has a shorter 3'-UTR apparently uses a polyadenylation signal closer to the stop codon, because it carries a different 5'-UTR, it is thus likely to be encoded by a different MT gene from the common carp genome. Another variant MT cDNA (clone 3–4) carries an identical 3'-UTR to clone 3–14 but it has a longer 5'UTR with an inserted sequence. Southern blot analysis of common carp genomic DNA also supported that there are at least three genes for MT in the genome of common carp. Constitutive expression of MT mRNA is detectable in liver, kidney, intestine and brain of carp tissues.

1520 METALLOTHIONEIN TRANSGENIC AND KNOCK-OUT MICE DO NOT HAVE ALTERED SENSITIVITY TO ADRIAMYCIN TOXICITY

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Pretreatment of animals with bismuth or zinc protects against the toxic side effects of adriamycin, presumably by the induction of metallothionein (MT) in heart. This study was designed to use MT-I transgenic mice as well as MT-I and II knock-out (MT-null) mice to determine the role of MT in cellular resistance to adriamycin. Adriamycin (25–45 μmol/kg, ip) produced a dose-dependent lethality in both control and MT-I transgenic mice. A dose of 35 μmol/kg of adriamycin was lethal to 80-90% of control mice within 20 days. This dose of adriamycin produced similar lethality with average survival times in MT-I transgenic mice and MT-null mice. MT and glutathione levels in the hearts of MT-I transgenic mice were about three-fold and 50% higher than those in control mice, respectively. However, these MT-I transgenic mice were not protected against adriamycin-induced cardiotoxicity, as assessed by microscopic indices and by cardiac lipid peroxidation, as indicated by malonaldehyde and 4-hydroxy-2-(E)-nonenal assays. These results indicate that constitutive MT levels do not affect the sensitivity of animals to the toxic effects of adriamycin. (Supported by NIH Grant ES-01142)

1521 THE COPPER AND CADMIUM HOMEOSTASIS GENE, *BSD2*, IS LOCALIZED TO THE ENDOPLASMIC RETICULUM MEMBRANE IN YEAST

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Yeast cells lacking superoxide dismutase (SOD) are sensitive to oxygen toxicity. This defect can be reversed by a single mutation in the *BSD2* gene. Loss of *BSD2* function renders cells hypersensitive to copper and cadmium ion toxicity and also increases accumulation of these metals in yeast. The *B S D2* suppression of SOD deficiency presumably results from the hyper-accumulation of redox active copper ions. *BSD2* encodes a novel protein without any known metal binding motifs. However the BSD2 polypeptide does contain a number of Tyr-Tyr pairs and a single Cys residue, both of which can serve as potential metal binding ligands. We used site-directed mutagenesis to address how BSD2 functions as a metal homeostasis protein. Substitution of a centrally localized Tyr^{131}-Tyr^{132} pair with Phe-Phe abolishes the ability of *bsd2* mutation to suppress SOD defects, whereas substitution of the single Cys has no effect. *BSD2* encodes a heavily glycosylated membrane protein with a endoplasmic reticulum (ER) localization signal. We further demonstrate that BSD2 is localized to the ER by subcellular fractionation and indirect immunofluorescence microscopy. BSD2 may thus serve as a channel or a pump of copper and cadmium in the ER and thereby regulate the total cellular level of these metals. The mechanisms are under investigation. Supported by NIH training grant ESO7141 and NIH grant ROIGM 50016 awarded to V.C.

1522 Cu(II) INTERACTIONS WITH AN EXPERIMENTAL ANTI-HIV AGENT, 1-DEOXYNOJIRIMYCIN, AND OXYGEN ACTIVATION BY RESULTING COMPLEXES

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1-Deoxynojirimycin (DNJ), a potent iminosugar inhibitor of a-glucosidase I, has been recently used as an experimental anti-HIV agent. Potentiometric and spectroscopic studies of the complexation of DNJ by Cu(II) were performed at 25°C and at ionic strength of 0.1 M (KNO_3) in order to obtain thermodynamic and structural background for further studies of potential anti-HIV activity of resulting complexes. Effect of histidine, a physiological Cu(II) carrier, on coordination equilibria was also studied. Results indicated that DNJ forms two very stable complexes at physiological pH, $CuH_{-1}(DNJ)_2^+$ and $CuH_{-2}(DNJ)_2$, involving Cu(II) chelation by the N-5 and O-6 donor atoms. In the presence of histidine, ternary complexes are also formed, of which the CuDNJHis species is stable in the physiological pH range. Binary Cu(II)-DNJ complexes are extremely effective mediators of oxidation of dG to 8-oxo-dG by ambient O_2 and H_2O_2 at pH 7.4 (phosphate) and 37°C. DNJ does not influence Cu(II)-mediated DNA cleavage, but strongly enhances Cu(II)-mediated 8-oxo-dG production in DNA. The results suggest that Cu(II) may be able to enhance and broaden the anti-HIV activity of DNJ.

1523 CADMIUM INDUCED INACTIVATION OF HIV-1 APPEARS TO BE BASED IN ITS INTERACTION WITH THE ZINC-FINGERS OF THE VIRAL NUCLEOCAPSID PROTEIN

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The nucleocapsid (NC) protein of the retroviruses contains one or two highly conserved zinc-finger domains of the CysCysHisCys configuration. The incorporation of zinc into the zinc-fingers is essential for the proper folding of the NC protein. The integrity of these zinc-finger domains is required for the packaging of the viral RNA into the virion. It is also possible that the NC protein may have an active role during reverse transcription and/or other steps of the early phase of infection. Cadmium (Cd), a highly toxic metal and known carcinogen, can often substitute for zinc in zinc binding proteins. The NC protein's affinity for Cd is very similar to that of zinc. To determine the effects of Cd on the production of an infectious human immunodeficiency virus (HIV-1), virus producing cells were treated with Cd and virus infectivity was assayed. Five and 10 μM Cd reduced the virus infectivity by 30% and 80% of that of the untreated control, respectively. The production of the viral proteins was not significantly altered at these concentrations of Cd, nor was the activity of the *pol* gene products, protease and reverse transcriptase. Examination of the NC protein by non-reducing SDS-PAGE indicates that it forms multiple intermolecular disulfide bonds more readily as a result of Cd treatment. This data suggests that the interaction of Cd with zinc-finger proteins may result in the formation of disulfide bonds between the Cys residues of the zinc-fingers, inactivating the protein. This may provide some insight to the general interaction of Cd with cellular zinc-finger proteins and its effects on genetic expression. Research sponsored by the NCI under contract with ABL.

1524 RELATIONSHIP BETWEEN AN IRON DEFICIENCY ANEMIA AND RENAL ANEMIA AFTER ORAL CADMIUM ADMINISTRATION IN RATS

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Male Wistar rats (6 weeks old) were fed with a different iron (Fe) diet (Fe deficient diet: 7.5 ± 0.9 μg, Fe normal diet: 102.7 ± 6.1 μg). Cd (as CdCI2) was given at a dose of 0, 2, 10, and 30 mgCd/kg by a gastric tube daily for 6 consecutive days for 10 weeks. The relationship among an Fe deficiency, the renal dysfunction and plasma erythropoietin (EPO) was investigated in the manifestation of Cd-induced anemia. The plasma Fe concentration decreased in Fe deficient rats after oral Cd administration. Renal dysfunction, reflecting by the increased urinary excretion of enzymes (NAG,A AP,GST), was observed markedly in Fe deficient rats. Also the decrease of Hb and Ht by Cd toxicity was prominent in Fe deficient rats. However the compensatory elevation of plasma EPO was observed in animals induced anemia by Cd. The concentration of non metallothionein-Cd (non MT-Cd) in the kidney was higher in Fe deficient rats than that of Fe normal rats. In conclusion, the anemia induced by Cd toxicity may be relating primarily the Fe deficiency rather than the renal dysfunction resulted an insufficient production of EPO by Cd toxicity. (The present study is supported in part by the grant from the Ministry of ESSC and EA of Japan)

1525 TITANIUM OXIDE (TiO_2) AND CADMIUM OXIDE (CdO) INHIBIT NITRIC OXIDE (NO) PRODUCTION IN CULTURED MURINE MACROPHAGES

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NO is a reactive intermediate that serves important roles in cell signaling and macrophage-mediated defense against various pathogenic microbes and tumorigenesis. In order to understand mechanisms underlying host responses to wear debris particles from implanted metallic prostheses and metal particles of environmental concern, we examined the effects of TiO_2 and CdO on NO production in RAW 264.6 murine macrophages. Subconfluent cultures (1 x 10^5 cells) were incubated for 48 hr prior to adding particles. Medium was changed and cells were exposed to particles alone (TiO_2-0.1, 0.2, or 0.5 mg; CdO - 1, 6.25, 12.5, 25, or 50 μg), LPS (*E. coli*) alone, or particles + LPS for 48 and 72 hr. NO levels were assessed by incubating aliquots of medium with Griess reagent, which measures formation of nitrite. Neither TiO_2 or CdO alone stimulated NO production. LPS, a known macrophage activator and inducer of NO, stimulated NO production. TiO_2 and CdO inhibited the

LPS-induced production of NO in a concentration-dependent manner at 48 and 72 hr after exposure. The data suggest that metallic oxides may disrupt NO production in macrophages. This effect may contribute to the pathophysiology of various diseases by compromising host defense mechanisms and cell signaling.

1526 CADMIUM PERTURBS CALCIUM HOMEOSTASIS IN RAT OSTEOSARCOMA (ROS 17/2.8) CELLS

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The mechanism of the toxic effects of Cd^{2+} on normal bone growth and bone cell function is not completely understood at this time. However, Cd^{2+} does perturb the cytosolic free Ca^{2+} concentration and directly activate protein kinase C (PKC) in ROS 17/2.8 cells. PKC is activated by Ca^{2+} and mediates Ca^{2+} metabolism. Therefore, cadmium may cause changes in steady state Ca^{2+} metabolism which is normally precisely controlled. Such changes would have implications for cell functions mediated by the Ca^{2+} messenger system. The purpose of this study was to characterize the effect of Cd^{2+} on Ca^{2+} metabolism in ROS 17/2.8 cells. Cells were labeled with ^{45} Ca (1.87 mM Ca) for 20 hr in the presence of 0.01 μM, 0.1 μM, or 1.0 μM Cd^{2+}. Following an EGTA rinse, kinetic parameters were determined from ^{45}Ca efflux curves. Three kinetic compartments described the intracellular metabolism of ^{45}Ca. Cadmium caused a large (approximately 10X) dose-dependent increase in Ca^{2+} flux across the plasma membrane and an accompanying decrease in the most rapidly exchanging intracellular Ca^{2+} compartment (S_1). This change indicates an increased efflux of calcium from ROS 17/2.8 cells. However, there was no change in total cell Ca^{2+} because flux between S1 and the intermediate Ca^{2+} compartment (S_2) was also increased and S_2 increased significantly. This data suggest that Cd^{2+} is perturbing steady state calcium metabolism by increasing Ca^{2+} efflux from the cell and by causing redistribution of Ca^{2+} within the cell. This work is supported by NIH grant ES06087.

1527 INTERACTIONS OF NICKEL(II) WITH HISTONES. ENHANCEMENT OF 2'-DEOXYGUANOSINE OXIDATION BY Ni(II) COMPLEXES WITH CH_3CO-CYSALA-ILE-HIS-NH_2 (CAIH), A MODEL OF THE BINDING SITE OF HISTONE H3

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The mechanisms of carcinogenesis by nickel, a human carcinogen, may include mediation of promutagenic oxidative damage through Ni(II) binding to chromatin components, including histone H3 [Bal, W. et al., *Chem. Res. Toxicol.* 8: 683-692, 1995]. To further test this hypothesis, studies of 2'-deoxyguanosine (dG) oxidation by H_2O_2 in the presence of CAIH and/or Ni(II) have been carried out in 100 mM phosphate buffer, pH 7.4 at 37°C. The dimeric CAIH oxidation product, CAIH disulfide, and its weak, octahedral Ni(II) complex, rather than the monomeric CAIH and its strong, square planar Ni(II) complex were found to be major catalysts of 8-oxo-dG formation. The complexes were much more active than the ligands alone, especially at submillimolar H_2O_2. The reaction was found not to involve detectable amounts of free radicals or Ni(III). These results support the hypothesis and also indicate that molecular mechanisms of nickel carcinogenesis may involve oxidative damage processes catalyzed by weak Ni(II)-peptide disulfide complexes.

1528 ELIMINATION OF INGESTED ALUMINUM IN THE BILE OF UNANESTHESIZED RATS

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We evaluated the importance of bile as an excretory route for orally-administered aluminum. Bile ducts in 30 male Sprague-Dawley rats were cannulated to allow both bile collection and reinfusion of bile acids. Five days following surgery, rats were given a single oral dose of aluminum (0, 0.2, 0.4, or 0.8 mmol) as aluminum lactate in 1 ml of 16% citrate by gavage. Bile was collected 1–7 hr after dosing from conscious rats. Biliary aluminum excretion was highest 1–2 hr after dosing which probably reflected first pass hepatic elimination of aluminum from the portal circulation. Rats dosed with 0.2, 0.4, or 0.8 mmol aluminum excreted significantly higher total amounts of aluminum in bile (2.73 ± 0.43 μg; 1.99 ± 0.29 μg; and 2.80 ± 0.93 μg, respectively) than did the control rats (0.34 ± 0.03 μg). Biliary aluminum excretion did not differ among animals given aluminum suggesting that this process was saturated at these doses. Rats dosed with 0.8 mmol aluminum retained significantly greater amounts of aluminum in serum, liver, spleen, and kidney than those given 0.2 or 0.4 mmols. This suggests that physiological mechanisms that protect against soft tissue aluminum accumulation and retention of aluminum in plasma were overwhelmed in animals given the 0.8 mmol dose. Supported by Coll. of Agriculture & Life Sci. Project 2623 and NIH DK41116.

1529 OXIDANT-PRODUCING ACTIVITY OF FE(II) IN TISSUE CULTURE MEDIA

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Ferrous iron (Fe^{2+}) is able to produce oxidants and may play an important role in diverse pathologies such as tissue aging and cancer. Using electron spin resonance and spin trapping technique, we investigated the role of cell culture medium compositions, and pH, on the formation of oxidants produced by Fe^{2+} and O_2. We found that oxidant formation in distilled water increased proportional to the concentrations of Fe^{2+} from 50 μM to 5 mM, and then sharply decreased to zero as the concentration of Fe^{2+} reached 50 mM. This phenomena was observed in several tested cell culture media, and oxidant-producing activity for a given concentration of Fe^{2+} was in an increasing order as follows: complete α-MEM < bicarbonate buffer < distilled water < 10 mM of PBS < 50 mM of PBS < 250 mM of PBS. The concentration of Fe^{2+} which gave the maximal oxidant-producing activity was shifted higher in proportion to the concentration of PBS, i.e. from 5 mM of Fe^{2+} to 50 mM when PBS was increased from 10 mM to 250 mM. The disappearance of free radical adducts at higher concentrations of Fe^{2+} is probably due to the low pH and low oxygen diffusion in the tested media, which can significantly slow down the oxidative reactions. Our studies strongly indicate that pH of the cell culture medium plays a determinant role in Fe^{2+}-catalyzed oxidant formation, and should be taken into account when testing Fe^{2+} compounds in biological systems. This work was supported by NIOSH OH03253.

1530 GADOLINIUM CHLORIDE-INDUCED SITE-SPECIFIC MINERALIZATION OF THE STOMACH IN RATS

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Studies have identified interstitial, fundic gastric mineralization in rats exposed intravenously to $GdCl_3$. To determine the relationship of gastric mineralization to changes in circulating calcium and phosphorus levels, groups of 5 male Sprague Dawley rats were injected intravenously with 0.07 mmol/kg $GdCl_3$ or saline and euthanized on Day 3. Ten of the rats exposed to saline or $GdCl_3$ were fasted 16 hr prior to dosing to determine the effects of fasting on ensuing histomorphologic changes. The stomachs were fixed by injection of neutral-buffered formalin into the stomach lumen and the entire stomach of each rat was embedded in paraffin, sagittally sectioned at 5 micrometers and stained with H & E and Von Kossa's stain. This procedure allowed for the examination of the entire contiguous stomach mucosa. H & E stained sections of stomachs from 10/10 rats exposed to $GdCl_3$ were characterized by a linear band of patchy, minimal-to-mild degeneration and minimal necrosis of the cells lining the upper one-third of the neck of the gastric glands. Von Kossa stained sections revealed a narrow, linear band of interstitial mineral deposited in this same region. Mineral deposition was frequently present in areas that lacked clear evidence of cellular degeneration or necrosis and was restricted to the parietal cell-rich, fundic portion of the glandular stomach; the pyloric area, which lacks parietal cells, was devoid of mineralization. Fasting of rats for 16 hr prior to dosing did not alter the incidence or severity of the observed lesions. X-ray microanalysis of the mineralized band in previous studies indicated that such gastric mineral deposits contained mostly Ca, P and some Gd. Total plasma calcium and phosphorus levels in earlier studies were increased but ionized calcium levels in this study were unchanged. The mechanism for Gd-induced mineralization of the gastric interstitium in the vicinity of parietal cells is unknown but it is speculated to be due to local perturbation of Ca-dependent parietal cell function in conjunction with elevated total plasma calcium levels.

1531 EFFECTS OF INTRAVENOUS GADOLINIUM CHLORIDE ON CIRCULATING CALCIUM AND PHOSPHORUS LEVELS IN THE RAT

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The intravenous administration of $GdCl_3$ to rats results in a discrete band of mineralization of the fundic glandular gastric mucosa. This mineral band is composed of calcium and phosphorus. To investigate the relationship of gastric mineralization to changes in circulating calcium and phosphorus levels, male Sprague Dawley rats were intravenously injected with 0.07 mmol/kg $GdCl_3$ and euthanized at intervals from 5 min to 48 hrs post dose. Plasma total calcium and phosphorus were measured, and stomach sections were processed for microscopic evaluation of mineral deposition. Plasma total calcium levels increased approximately 2-fold within 3 hrs, remained elevated for approximately 12 hrs and declined to control levels by 48 hrs post-dose; plasma phosphorus levels approximated the changes in total calcium. Mineralization was noted in fundic stomach sections 6 hrs post-dose, progressing in severity to 48 hrs. Additional studies showed the increase in total calcium produced by $GdCl_3$ administration was not reflected by changes in either the physiologically active ionized calcium pool or changes in albumin levels, suggesting the albumin-bound calcium pool was not affected. Urinary excretion of calcium and phosphorus were not affected after $GdCl_3$ administration nor was renal impairment indicated, since creatinine levels were normal. Lastly, the increases in calcium or phosphorus levels and the occurrence of the lesion were unaffected by either feeding or fasting the animals prior to $GdCl_3$ administration. These results suggest the intravenous injection of $GdCl_3$ to rats increases plasma total calcium and phosphorus levels, which temporally related to gastric mineralization. Ionized calcium and albumin levels are not affected, suggesting that the increased calcium levels are in the complexed calcium pool (e.g., citrate). The causal relationship of increased calcium and phosphorus levels to gastric mineralization is presently unknown.

1532 LACK OF TOXICITY OF CHROMIUM CHLORIDE AND CHROMIUM PICOLINATE

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Trivalent chromium is considered relatively nontoxic and an oral reference dose of 1 mg/kg body weight has been established by expert panels of the U. S. Environmental Protection Agency. However, this value has been questioned due to the study of Meenakshi et al. (Indian J. Environ. Hlth. 31:250–256, 1989) who reported toxicity of trivalent chromium in rats give 10 mg of chromium as chromium chloride per kg body weight by gavage. In light of these findings, we evaluated the toxicity of chromium chloride and a more bioavailable form of trivalent chromium, chromium tripicolinate. Harlan Sprague Dawley rats (4 weeks of age) were fed a stock diet to which was added 0, 5, 25, 50 or 100 μg of chromium per gram of diet as chloride or picolinate for 24 weeks (highest levels are roughly 30 mg/kg body weight). There were no statistical differences in body weight or blood variables among all the groups tested at 11, 17 and 24 weeks. Histological evaluation of the liver and kidney of control animals fed 100 ppm chromium chloride or picolinate also did not show any detectable differences. Liver and kidney chromium concentrations increased linearly for both the chloride and picolinate fed animals. Animals consuming the picolinate supplemented diets had 2 to 3 times higher chromium concentrations in both the liver and kidney than those fed chromium chloride. These data demonstrate a lack of toxicity of trivalent chromium, at levels that are on a per kg basis, more than 10,000 times the upper limit of the estimated safe and adequate daily dietary intake for humans.

1533 INTERACTIONS OF ARSENICALS WITH PROTEINS IN RAT LIVER CYTOSOL

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Protein-arsenical interactions were examined in an *in vitro* methylation system that contained rat liver cytosol, glutathione, S-adenosylmethionine and 1 μM [^{73}As] arsenite. After incubation at 37°C for up to 90 minutes, low molecular weight components of the assay system were removed by ultrafiltration and cytosolic proteins separated by size-exclusion chromatography on Sephacryl S-300 gel. Four [^{73}As]-labeled protein peaks were identified. Approximate masses of these peaks were: A- ≥ 1500 kDa, B-146 kDa, C-37 kDa, and D-12 kDa. Protein-bound [^{73}As] released by CuCl treatment was speciated by thin-layer chromatography. The amounts and the ratios of the arsenicals in each peak depended upon the incubation interval. Inorganic As was present in all protein peaks. Methyl As (MAs) was mostly associated with peaks B and D; dimethyl As (DMAs), mostly with peaks C and D. To examine the effect of valence on organoarsenical binding, trivalent or pentavalent ^{14}C-MAs or ^{14}C-DMAs was incubated with liver cytosol. Proteins in peaks A, B and C bound preferentially trivalent MAs and DMAs. Peak D bound both trivalent and pentavalent MAs and DMAs. Thus, trivalent organoarsenicals, the major products of As biomethylation, interact with several cytosolic proteins. (This abstract does not necessarily reflect EPA policy.)

1534 *IN-VITRO* METABOLISM OF INORGANIC ARSENIC BY RAT CAECA AND MICRO FLORA

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This study examined the qualitative and quantitative aspects of the metabolism of inorganic arsenic by rat caeca and microflora. Caeca with contents from adult male Fischer 344 rats were harvested and homogenized under a blanket of CO_2 and transferred into an anaerobic chamber containing an atmosphere of 85% nitrogen, 10% hydrogen, and 5% CO_2. Homogenization and culture media (gelatin-free VPI buffer that contained 1 mg/ml L-cysteine) was used to prepare a 28% (w/w) homogenate. This media maintains the viability of microorganisms without supporting significant population growth (i.e., <2X). Aliquots of homogenate (100 ul) were pipetted into autosampler vials containing 200 ul inserts and sealed with silicone /PTFE lined snap caps which maintained an anaerobic atmosphere. [^{73} As] Arsenate or [^{73} As] arsenite (1 uCi) was injected into the vials, including control vials that contained medium without homogenate, and vials were incubated at 37° C in a shaking water bath. Duplicate vials were taken after 3, 6, 21, or 45 hours of incubation. Dose-effect vials were taken after 21 hours. Samples were treated with 0.1 M CuCl (pH 1) to liberate protein-bound ^{73}As. Radiolabelled As-species were analyzed in 10 kDa ultrafiltrates from CuCl treated samples by TLC. For both arsenate or arsenite treated samples, the major organoarsenical product was monomethyl As (MMAs). At 21 hours of incubation, MMAs accounted for 42% of the total ^{73}As in samples incubated with arsenate and about 35% in samples incubated with arsenite. For each inorganic arsenic species, less than 5% of the total ^{73}As was converted to dimethyl As (DMAs). Over the concentration range of 0.1 to 10 uM As, MMAs was always the major metabolite. There was no significant effect of substrate concentration on the percentage of As converted to MMAs. The absence of DMAs in this in-vitro assay system suggests that the assay conditions do not support dimethylation, that the methyltransferase involved in As dimethylation is absent or there is an endogenous inhibitor present in the assay system. (This abstract does not necessarily reflect EPA policy)

1535 GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) DYNAMICS DURING THE EVOLUTION OF ORGANOPHOSPHORUS ESTER-INDUCED DELAYED NEUROPATHY (OPIDN)

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Alteration in tissue concentration of GFAP, the major intermediate filament protein of astrocytes, has been used as a bioassay reflecting dose, time and regional effects for a variety of neurotoxicants (O'Callaghan, Ann NY Acad Sci 679: 195). We now extend these studies to assess changes in spinal cord GFAP during the progressive clinicopathologic events in OPIDN. The latter was induced in adult White Leghorn hens by a single im dose of 2.5 mg/kg phenyl saligenin phosphate. Clinical signs of ataxia and weakness began on post-dosing day 7, and reached a deficit score of 2 (using a 0-4 scale with controls = 0) on day 10 and 4 by day 14. Cohorts (n = 4) were euthanized on days 4, 8, 14, and 21, and cervical spinal cord collected for microtiter plate sandwich ELISA (O'Callaghan, Neurotox. Teratol. 13: 275), immunocytochemistry for GFAP, and routine (hematoxylin and eosin staining) histopathology. With the latter, degenerating nerve fibers were seen in the rostral levels of the spinocerebellar tracts, beginning on day 14, and were more advanced on day 21. ELISA revealed that control hen spinal cord had 4.38 ± 0.24 μg GFAP/mg protein (mean ± SE, n = 4). Significant decreases in GFAP were noted on days 4 and 8 (1.10 ± 0.30 and 1.87 ± 0.04 μg/mg protein

respectively), whereas on days 14 and 21 significant increases were observed (5.59 ± 0.47 and 5.92 ± 0.93 μg/mg protein, respectively). Semiquantitatively scored changes in immunocytochemically detected GFAP-positive reactive astrocytes were not as definitive. This study demonstrates dynamic changes in spinal cord concentration of GFAP during the evolution of OPIDN, with an initial depression being followed by an elevation of this astrocytic protein.

1536 NEUROPATHOLOGY OF ORGANOPHOSPHORUS ESTERINDUCED DELAYED NEUROPATHY (OPIDN) IN HENS. LESIONS OF SPINAL CORD GRAY MATTER REVISITED

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Classical neuropathologic studies of the hen model of OPIDN focused on dramatic myelinated fiber distal degeneration in peripheral nerve and long cerebrospinal tracts (Cavanagh, J Neurol Neurosurg Psychiat 17:163). More recently, investigators using reduced silver impregnation methods have drawn attention to prominent gray matter lesions (Tanaka and Bursian, Brain Res 484:240). The latter were stated to consist of degenerating axons and their terminals. The present study was conducted to determine if ultrastructural correlates of these lesions could be detected in the hen OPIDN model. Spinal cord from archival tissue blocks from hens with advanced clinical OPIDN (21 days after a single im dose of 2.5 mg/kg phenyl saligenin phosphate) was prepared for transmission electron microscopy. Bilateral distal nerve fiber degeneration of long myelinated tracts, typical of OPIDN, was noted. Axonal lesions (swelling, membranous arrays, collapse) were also seen in gray matter ventral horns, affecting varying sized myelinated fibers and extending to their presynaptic terminals. Such changes had been noted in earlier ultrastructural studies of spinal cord gray matter in OPIDN (Bischoff, Acta Neuropath 15:142; Prineas, J Neuropath Exp Neurol 18:571), and are likely nidi for degenerating fiber silver impregnation described by Tanaka and Bursian. In addition, scattered dendrites containing enhanced membranous arrays were noted, suggesting a postsynaptic neuropathic event in OPIDN. (supported in part by US EPA 68D80098)

1537 INVESTIGATION OF DELAYED NEUROTOXICITY POTENTIAL IN HENS WITH NALED

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White leghorn hens were given technical naled (1,2-dibromo-2,2-dichloroethyl dimethylphosphate) daily by gavage for 28 days at dosages of 0.5, 2 and 4 mg/kg. A vehicle control and two positive control (35 and 45mg TOCP/kg/day) groups were included. Clinical signs and locomotor ability were monitored for an additional 21 days.

Satellite groups were sacrificed 2 days after the last dose for determination of acetylcholinesterase (AChE) and neurotoxic esterase (NTE) in brain and spinal cord. After the 21-day observation period, hens were perfused and necropsied. Brain, spinal cord and sciatic and tibial nerves were examined microscopically. Transient reductions in body weight and feed consumption occurred in the 4 mg/kg/day group. Reduced brain AChE activity levels in the naled groups appeared dose related, but NTE levels were not adversely affected. There were no treatment related clinical signs, locomotor effects, or histopathological findings in the naled groups. TOCP groups showed clinical signs, microscopic lesions and dose related effects on AChE and NTE, characteristic of organophosphate induced delayed neuropathy. Thus, naled was found negative for delayed neurotoxicity.

1538 NEUROFILAMENT PROTEIN AND ERYTHROCYTE SPECTRIN CROSS-LINKING PRODUCED BY INHALATION OF CS_2

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Previous studies have demonstrated the ability of CS_2 to covalently cross-link proteins as a potential mechanism of toxicity. In the present investigation the utility of erythrocyte spectrin cross-linking for evaluating exposures to CS_2 and the role of CS_2 mediated neurofilament cross-linking in the induction of CS_2 neuropathy were examined. Rats were exposed to CS_2 by inhalation and neurofilament protein and erythrocyte spectrin cross-linking analyzed as a function of exposure level and duration. Light microscopic examination of spinal cords revealed axonal swellings at 8 weeks exposure for the 500 and 800 ppm levels. Spectrin cross-linking exhibited a cumulative dose response with significant increases in spectrin dimer over control values evident for the two neurotoxic levels and the one subneurotoxic level (50 ppm) after 2 weeks and 8 weeks exposure, respectively. Covalent cross-linking of neurofilament proteins involved all three protein subunits and was demonstrable at neurotoxic and subneurotoxic levels of exposure. For the two neurotoxic levels of exposure, neurofilament protein cross-linking occurred prior to axonal structural changes. These results support neurofilament cross-linking as a contributory mechanism in the CS_2 mediated accumulation of neurofilaments and suggest that spectrin dimer formation may provide a sensitive preneurotoxic biomarker paralleling the pathological event ocurring in the axon.

1539 ULTRASTRUCTURAL CHANGES IN THE PERIPHERAL NERVOUS SYSTEM (PNS) OF MICE ADMINISTERED PACLITAXEL (TAXOL®) OR DOCETAXEL (TAXOTERE®)

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Paclitaxel and docetaxel are members of a new and novel class of antineoplastic agents, the taxanes, which act to stabilize microtubules. A sensory neuropathy occurs in some patients receiving taxane therapy. This study compared docetaxel- and paclitaxel-induced PNS ultrastructural changes. Mice received equimolar doses of paclitaxel (18 mg/kg) or docetaxel (17 mg/kg), iv, SID x 5d. Mice were euthanized 7d after the last treatment and perfused with McDowell Trump fixative. The sciatic nerve and lumbar dorsal root ganglia were embedded in epoxy resin, and semi-and ultra-thin sections were evaluated. PNS lesions were more severe in docetaxel- as compared to paclitaxel-treated mice, similar to that observed clinically. Taxane-related changes in the dorsal root ganglia consisted of cell body chromatolysis; others had increased phagolysosomes in the perikaryon. Prominent axonal swellings adjacent to affected cell bodies, increased satellite cells, and atrophy of myelinated axons were also seen. Axonal atrophy and drop out, associated with irregularly thickened and redundant myelin sheaths, were observed in the sciatic nerve. Accumulation of myelin membranes and lipid globules were present in some Schwann cells. Our findings suggest that taxane-induced peripheral neurotoxicity primarily involves cell body injury with secondary axonal changes.

1540 INHIBITION OF PHENYL VALERATE ESTERASES (PVE) SENSITIVE TO MIPAFOX AND PROMOTION OF ORGANOPHOSPHATE INDUCED POLYNEURO-PATHY (OPIDP)

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Certain esterase inhibitors exacerbate OPIDP (promotion). The target for OPIDP is neuropathy target esterase (NTE) defined among PVE resistant to paraoxon by inhibition with mipafox (50 μM). The target for promotion is not NTE although all promoters are NTE inhibitors. Hen brain, spinal cord and sciatic nerve homogenates were incubated (20 min, 37°C, pH 8.0) with paraoxon (40 μM) and mipafox (0–1 mM). Log-plots of remaining activities identified NTE_1, sensitive to 50 μM mipafox (*i.e.* standard NTE, I_{50} = 7 μM) and NTE_2 resistant to 50 μM and sensitive to 1 mM mipafox (I_{50} = 250 μM). NTE_2 corresponds to about 10, 20 and 30% of NTE_1 activity in brain, spinal cord and sciatic nerve, respectively. I_{50}s for NTE_1 and NTE_2 of promoters phenylmethanesulfonyl fluoride (PMSF) and n-butanesulfonyl fluoride (BuSF) were about 100 and 60 μM, respectively in all tissues. The I_{50}s of neuropathic diisopropylfluorophosphate (DFP) and di-n-butyl dichlorvos (DBDCVP) for NTE_2 were higher than those for NTE_1 (2–10 μM vs 0.2–0.5 μM and 60–200 nM vs 4–8 nM, respectively). About 70% inhibition of both NTE_1 and NTE_2 were obtained in hen nervous tissues with both PMSF (30 mg/kg, sc) or BuSF (5 mg/kg, iv) given at promoting doses. Inhibition of NTE_1 was 90% or higher whereas that of NTE_2 was 20–40% when 1 mg/kg sc of either DFP or DBDCVP were given at frankly neuropathic doses. These experiments indicate a correlation between inhibition of NTE_1 and initiation of OPIDP and that of NTE_2 and promotion.

1541 THE TEMPORAL AND SPATIAL PATTERN OF GAP-43 IMMUNOSTAINING IN THE DISTAL SCIATIC NERVE DURING AND FOLLOWING ACRYLAMIDE TREATMENT

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GAP-43 is a membrane-associated protein found at high levels in the developing nervous system and during regeneration from mechanical or chemical injury in the adult peripheral nervous system. Our laboratory has reported increased expression of mRNA and protein levels of GAP-43 following acrylamide treatment. In this study male Sprague-Dawley rats were administered 0.05% acrylamide in drinking water for 14 days, after which tap water was substituted. Clinical signs were evaluated and animals were sacrificed over an 80 day period from start of treatment. Signs declined from severe hindlimb splay and ataxia on day 14 to mild hindlimb splay on day 28 and no clinical signs of neurotoxicity by day 50. Sciatic nerve crush was used as a positive control, in a separate group of rats sacrificed 4 days after crush. Sections of distal sciatic nerves from treated and control animals were immunolabeled with anti-GAP-43 (91E12 monoclonal antibody). Control animals showed no evidence of GAP-43 labeling. Nerve crush increased the number of GAP-43 labeled neurites and sparsely-distributed in non-neuronal cells. Ten and 14 days after start of acrylamide treatment, GAP-43 labeled non-neuronal cells were increased, but the number of labeled neurites was not. By 28 days after start of the experiment there was an increase in both GAP-43 labeled neurites and non-neuronal cells within distal sciatic nerve. At 80 days GAP-43 staining was decreased in non-neuronal cells but remained present along regenerating neurites. These results suggest that acrylamide treatment, in contrast to nerve crush, induces GAP-43 production in non-neuronal cells prior to that in neurites. The lack of GAP-43 staining in neurites prior to cessation of acrylamide treatment may be due to insufficient degeneration to induce GAP-43 or suppression of neurite outgrowth by acrylamide.

1542 PERIPHERAL NEUROPATHY IN RATS TREATED WITH INTRAVENOUS PACLITAXEL

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Sensory neuropathy is a major toxicity associated with the clinical use of the antineoplastic agent, paclitaxel (TAXOL®). Four experimental groups were administered either paclitaxel (at 2.5 or 8.5 mg/kg/day), the vehicle for TAXOL® (a 1:1 mixture of Cremophor EL® and ethanol diluted in saline), or saline by intravenous injection once daily for a period of 5 days. The dosing period was followed by a 28-day test article-free observation period. Paclitaxel induced signs of generalized toxicity common to cytotoxic agents; however, no clinical evidence of neuropathy occurred. Histopathologic examination of the brain, sciatic nerve, spinal cord, and spinal nerve roots revealed Wallerian-like axonal degeneration and secondary demyelination in the sciatic nerve, the dorsal funiculi of the spinal cord, and dorsal spinal nerve roots. The severity and incidence of these changes were proportional to dose and were greater on Day 33 than on Day 8, indicating a lack of reversibility during this time period. The results of this study support the use of the rat as a model for the study of paclitaxel-induced sensory neuropathy in humans.

1543 ACRYLAMIDE NEUROTOXICITY IN A TRANSGENIC MOUSE MODEL LACKING AXONAL NEUROFILAMENTS

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A transgenic mouse model, expressing an NFH/galactosidase fusion protein and lacking axonal neurofilaments (Eyer and Peterson, Neuron 12:1, 1994), was used to test the validity of the neurofilament hypothesis in mammalian acrylamide neurotoxicity. Transgenic mice (T) or their nonafflicted littermates (NT) received daily i.p. injections of acrylamide (ACR; 50mg/kg), propionamide (Prop;50mg/kg) or saline following blind testing of rotorod performance and preliminary nerve morphology. A cumulative increase in number of animals failing the rotorod test was observed for all ACR-exposed mice; transgenics (n = 8) failed from day 10–17, NT littermates (n = 8) failed on day 12–18. Ataxia, footdrop and hindlimb paralysis were observed in all ACR-exposed mice. No behavioral changes were observed with any Prop (n = 16) or in saline-injected (n = 6) animals. Tibial nerves of ACR-exposed animals possessed axons with increased vesicles or axonal degeneration of comparable magnitude in transgenics and non-transgenics. We conclude that acrylamide alteration of neurofilaments is not a site of action necessary for the production of axonal degeneration.

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1544 EFFECTS OF *IN VITRO* ANOXIA AND POST-ANOXIA REOXYGENATION ON ELEMENTAL COMPOSITION OF PERIPHERAL NERVE FROM ACRYLAMIDE (ACR) EXPOSED RATS

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Previously we have shown that ACR, like HD, causes tibial nerve internodal axonal atrophy and a selective decrease in K (LoPachin and Lehning, *Neuro Toxicology* 15, 247–260, 1994). Loss of K might represent a direct effect of ACR or a homeostatic response to atrophy. To evaluate the significance of reduced internodal K, tibial nerves were obtained from rats treated with ACR (50 mg/kg/d x8d, ip) or vehicle and subjected to in vitro anoxic challenge (3 hr) and post-anoxic reoxygenation (2 hr). Nerves were incubated in a brain slice chamber and perfused with Ringer's solution aerated with 95% O_2/5% CO_2. Anoxia was induced with 95% N_2/5% CO_2. X-ray microanalysis was used to measure Na, K and Ca concentrations and % water. Anoxia elevated axoplasmic and mitochondrial Na and Ca and depleted K in nerves from control animals. ACR treatment protected axons from anoxic injury; i.e., Na and K deregulation was markedly reduced and Ca content remained normal. Post-anoxic reoxygenation completely restored normal elemental composition in control nerves. In contrast, reoxygenation of tibial nerve from ACR rats produced a mixed population of myelinated axons consisting of recovered axons and axons expressing continued elemental deregulation. Parallel normoxic control studies demonstrated that the above results were not due to extended (5 hr total) in vitro incubation. Results indicate in vivo exposure to ACR conveys axonal resistance to anoxic challenge. However, ACR appears to predispose certain axons to reoxygenation failure. Elucidation of how ACR produces these effects could provide insight into mechanisms of ACR neuropathy.

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1545 SUBCHRONIC TOXICITY AND NEUROTOXICITY OF N-METHYLPYRROLIDINONE (NMP) IN RATS

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The toxicity of NMP was evaluated by administration of diets containing 0, 3000, 7500, or 18000 ppm NMP to Crl:CD®BR male and female rats for approximately 90 days. Body weights and food consumption were measured weekly. Clinical pathology was evaluated at 45 and 90 days. Functional observational battery and motor activity were conducted at weeks 4, 8, 13, and after 4 weeks of recovery. A morphological evaluation was conducted at 13 weeks or after 4 weeks of recovery. Body weights, weight gains, and food consumption were decreased in males and females at 7500 and 18000 ppm. Males and females had a significantly higher incidence of bright yellow colored urine at all concentrations. There were no adverse effects on organ weights or morphology. Compound-related changes were observed in 3 neurobehavioral parameters. Foot splay was significantly increased in 7500 and 18000 ppm males. A higher incidence of low arousal and slight palpebral closure occurred in 18000 ppm males. These effects may be due to systemic toxicity since no compound-related morphological changes occurred in nervous tissue or in any of the other 33 neurobehavioral parameters. The NOAEL was 3000 ppm in both males and females.

1546 28-DAY REPEATED DOSE DELAYED NEUROTOXICITY STUDY IN HENS WITH REOFLAM PB-460 TRIARYL PHOSPHATE

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Reoflam PB-460, or tris(dibromophenyl) phosphate, is a triaryl phosphate compound used as a flame retardant. The potential of PB-460 to produce organophosphorus induced delayed neurotoxicity (OPIDN) was evaluated in an EPA guideline study. Groups of 11 White Leghorn hens received PB460, corn oil (negative control) or 35 mg/kg/day of tri-o-cresyl phosphate (positive control) via oral gavage for 28 consecutive days. Doses of PB-460 tested

were 250, 500 or 1000 mg/kg/day in corn oil. Clinical signs, body weights, and degree of ataxia were evaluated on a regular basis. At 24 hours after the final dose, 3 hens/group were sacrificed for brain and spinal cord neurotoxic esterase (NTE) determinations. The remaining animals were perfused and the central and peripheral nervous systems underwent pathological examination. Positive control hens exhibited clinical signs of OPIDN and had axonal degeneration in the spinal cord and tibial nerves. Brain and spinal cord NTE were also significantly inhibited in positive control hens. PB-460 did not produce clinical signs of OPIDN, toxicologically significant NTE inhibition or neuropathological lesions. Under the conditions of this study, PB-460 did not cause delayed neurotoxicity when administered to hens up to and including the limit dose of 1000 mg/kg/day via oral gavage for 28 days.

1547 REPEATED DOSING OF HENS WITH PHOSPHORO-THIOIC ACID O-(2-CHLORO- 2,3,3-TRIFLUOROCY-CLOBUTYL) O-ETHYL S-PROPYL ESTER (KBR-2822) CAUSING MARGINAL NTE INHIBITION DOES NOT INDUCE CLINICAL NEUROPATHY

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Certain esterase inhibitors such as KBR-2822 and phenylme-thanesulfonyl fluoride (PMSF) cause exacerbation (promotion) of toxic (2,5-hexanedione) and traumatic (nerve crush) axonopathies. The target for organophosphate neuropathy (NTE) is unlikely to be that of promotion and the hypothesis has been that promotion might possibly affect functions involved in the physiological turnover of damage and compensation/repair of the axons. Aim of study was to ascertain whether repeated treatment with KBR-2822 causes neuropathy as a result of unrepaired physiological axonal damage. Hens were treated with KBR- 2822 (0.2 or 0.4 mg/kg/day) by gavage for 90 days and observed for clinical signs (0–8 point scale) up to 21 days after treatment. No neuropathy was observed. Enzyme activities were measured at intervals and mean percentages of inhibition (number of animals) were: NTE: 7 ± 5 (9), 10 ± 7 (9) and 24 ± 12 (9) in the 0.2 mg/kg group and 13 ± 9 (9), 18 ± 9 (9) and 40 ± 12 (9) in the 0.4 mg/kg group in brain, spinal cord and peripheral nerve, respectively. AChE in brain was 15 ± 15 (9) and 48 ± 5 (9) in the two experimental groups. Diisopropyl-fluorophosphate (0.5 mg/kg, sc) neuropathy was promoted (from median score 1.5 to 8) by PMSF (120 mg/kg, sc) in both untreated and KBR-2822 pretreated animals. Lack of neuropathy after repeated treatment with KBR-2822 might be due to short duration of treatment, low dose or because physiological damage is minimal and not promotable.

1548 2,5-HEXANEDIONE DIRECTLY AFFECTS KINESIN-BASED MICROTUBULE MOTILITY

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Reductions in fast anterograde axonal transport (faAXT) by γ-diketones have been correlated with axonal degeneration (Sickles, Neurotoxicology 10:103, 1989). The effects of 2, 5-hexanedione on kinesin, the faAXT motor, were determined using video enhanced DIC microscopy to view the movement of taxol-stabilized microtubules (MT) over an absorbed bed of kinesin. Preincubation of kinesin with 2, 5-HD (37C; 20min) resulted in unsteady MT motility and an increase in MT detachment in controls from 6.7 ± 1.4% (n = 43) to 41.4 ± 4.2% (1mmM;n = 48)- 60.6 ± 4.8% (4mM;n = 44). No effect on rate of movement was observed (saline = 889 ± 43nm/sec; 1mM = 905 ± 23; 4mM = 859 ± 39). The effect was temperature dependent from 22–37C as well as neurotoxic specific; 2, 3-HD and 3, 4-HD did not change motility. Preincubation of MT with 2, 5-HD increased MT detachment from 5% (saline) to 19% (1mM)- 39% (4mM) without affecting rate. Acrylamide (0.7mM) was twice as effective as 2, 5HD (4mM) in altering kinesin driven MT motility. We conclude that covalent modification of kinesin and MT by 2, 5-HD contributes to reductions of faAXT which may lead to axonal degeneration. These data suggest a common protein of action of acrylamide and γ-diketones in producing axonal degeneration Supported by ES 05160.

1549 LASALOCID-INDUCED NEUROTOXICITY IN BROILER CHICKENS

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Lasalocid, a polyether, ionophorus anticoccidial agent, is widely used in the poultry industry to prevent and control coccidial infections. Previous reports indicate that this compound may cause a peripheral neurotoxicity in poultry. A feeding trial using 1x, 2x, 3 and 4x the recommended levels of lasalocid produced a dose-dependent increase in ataxia in Cobb-cross broilers. Ataxia was measured by a clinical scoring system. Onset of neurotoxicity was 7–10 days after introduction to the medicated feed in birds of 21 and 42 days of age. Histologic and ultrastructural evidence suggest specific damage to the peripheral nervous system (sciatic nerve). Electrophysiologic studies show a decrease in the nerve conduction velocity of the superficial peroneal nerves in lesioned as compared to normal birds. Ataxia was seen without significant muscle damage as measured by histopathology or clinical pathology (serum electrolytes and CPK, AST and LDH activities) evaluation. In contrast, two other ionophores, monensin and salinomycin, failed to produce ataxia similar to that caused by lasalocid.

1550 RAT SERUM WITH DETECTABLE NEUROTYPIC AND GLIOTYPIC AUTOANTIBODIES BLOCKS NEUROMUSCULAR ACTIVITY

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Autoantibodies to neuronal and glial antigens [NF68; NF160; NF200; GFAP] have been detected in humans and animals exposed to lead (Pb) or mercury. These autoantibodies have been shown to predict and correlate with neurotoxicity. Since these antigens are cytoskeletal proteins, it suggests that antibody production likely occurs after degeneration of the neural processes with which they are associated. This also suggests that autoantibodies to other cellular structures, like the synapse, may be produced. In the present study, male F344 rats were exposed to 450 ppm Pb (as acetate) for 7 weeks and sera were collected. Autoantibodies to NF proteins, GFAP and MBP were detected, by ELISA, in sera of Pb-exposed animals, but not in controls. Using a chick biventer cervicis nerve-muscle preparation, sera of rats exposed to Pb, but not control sera, significantly reduced muscle contraction produced by electrical stimulation. Sera from Pb-exposed rats did not effect contraction produced by exogenous acetylcholine (ACh). This suggests that neuromuscular activity was affected presynaptically and not post-synaptically. There were no detectable levels of Pb in the serum. Therefore, we hypothesize that autoantibodies produced against presynaptic proteins, possibly those involved in neurotransmitter release, may be responsible for reduced neuromuscular activity. (Sponsored by NIH Center Grant ES-00260).

1551 *IN VIVO* EXPOSURE TO 2,5-HEXANEDIONE (HD) COMPROMISES RAT TIBIAL NERVE MYELINATED AXON RECOVERY FROM *IN VITRO* ANOXIC CHALLENGE

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Previously we have postulated that peripheral nerve myelinated axons are not injured by in vivo exposure to HD based on the absence of injury-typic elemental deregulation. Rather, we found that HD administered either orally or intraperitoneally causes selective losses of axoplasmic K and water contents which might represent homeostatic adjustments to developing axon atrophy (LoPachin et al., *J. Neurochem.* 63, 2266–2278, 1994; Lehning et al., *Toxicol. Appl. Pharmacol.* In press). To assess injury status, in the present study, tibial nerves were obtained from rats treated with HD (400 mg/kg/d x20d, ip) or vehicle and subjected to in vitro anoxic challenge (3 hr) followed by reoxygenation (2 hr). Nerves were incubated in a brain slice chamber and perfused with Ringer's solution aerated with 95% O_2/5% CO_2. Anoxia was produced with 95% N_2/5% CO_2. X-ray microanalysis was used to measure % water and Na, K and Ca concentrations. Control and HD nerves exhibited an equivalent response to anoxia; axoplasmic and mitochondrial Na and Ca were increased and K was decreased. Post-anoxic reoxygenation fully reestablished normal elemental composition in control nerve axons and mitochondria. Nerves from HD rats displayed a partial restoration of axonal and mitochondrial Na, Ca and K concentrations following reoxygenation. Parallel normoxic control studies demonstrated the above results were not due to extended (5 hr total) in vitro incubation. Results indicate recovery from in vitro anoxia is impeded in nerves from HD-treated rats. Whether this represents a direct effect of HD or is related to the development of axonal atrophy is currently under investigation. Supported by NIEHS Grant RO1 ES03830.

1552 ELECTROPHYSIOLOGICAL, CLINICAL AND PERFORMANCE TESTS IN RATS WITH PYRIDOXINE-INDUCED SENSORY NEUROPATHY OR MIPAFOX/PMSF-INDUCED OPIDN

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The extent of sensory versus motor fiber damage in organo-phosphorus compound-induced delayed neurotoxicity (OPIDN) is incompletely characterized. We used electrophysiological methods to characterize the caudal compound nerve action potential (cCNAP) using distal and proximal stimulation of the cCNAP to differentiate sensory and motor nerve components, respectively. Coordination was assessed using an accelerating rotorod and changes in gait and sensorimotor function were evaluated using a clinical grading scale. Treatments were pyridoxine (B6; 800 mg/kg/day ip × 6 days), mipafox (45 mg/kg ip) followed by the OPIDN potentiator phenylmethylsulfonyl fluoride (PMSF, 60 mg/kg sc) 24 hours later, or the appropriate solvent. Data were analyzed by repeated-measures ANOVA/Bonferroni-corrected t-test ($p<0.05$ = significant). B6 caused significant decreases in the distal cCNAP area and amplitude of 19 and 45%, respectively, on day 7 and 81 and 91%, respectively, on day 11. In contrast, no significant decreases in the distal cCNAPs were seen in rats given mipafox/PMSF or vehicles. B6 caused ataxia, hindlimb/forelimb splay, hindlimb crossing and loss of the righting reflex by day 7; these effects became severe by day 11. Mipafox/PMSF caused ataxia, hindlimb crossing and altered vocalization by day 15. B6 rats had decreased rotorod performance on day 3 and were unable to perform this test thereafter. Mipfox/PMSF rats had markedly decreased rotorod performance by day 15. B6-induced changes in the cCNAP were not observed in rats given mipafox/PMSF by day 15, though these rats showed clinical effects and decreased rotorod performance. Reports of sensory tract lesions in the spinal cord of mipafox/PMSF treated rats may explain the observed clinical/performance effects. Future studies will seek to characterize further the sensory effects of OP compounds on the rat peripheral nervous system.

1553 ACRYLAMIDE-REGULATED NEUROFILAMENT EXPRESSION IN RAT PHEOCHROMOCYTOMA (PC12) CELLS: A POSSIBLE MECHANISM OF NEUROTOXICITY

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Aberrant neurofilament accumulation is central to the pathogenesis of acrylamide-induced neurotoxicity. One possible mechanism for such accumulation is through the regulation of neurofilament gene expression by acylamide. In previous studies using the PC12 model system, we have found that nerve growth fator (NGF) receptor activation is required for NGF- but not for acrylamide-stimulated neurofilament protein synthesis. These results led to the hypothesis that acrylamide may act through certain downstream signaling steps that eventually regulate the expression of neurofilament genes. Thus, we tested our hypothesis by using [^{32}P]labeled cDNA probes specific for each neurofilament subunit (light NFL, medium NFM, and heavy NFH) and measuring their mRNA levels by Northern hybridization. Acrylamide and NGF alone caused up to 3.5-fold increase in NFM mRNA level, and their combination resulted in a 7-fold increase. While NFL mRNA level was increased by NGF up to 2-fold, it was decreased about 50% by acrylamide alone. However, their combined treatment resulted in a synergetic increase up to 10-fold. The NFH mRNA level was decreased 50% and 80% by NGF and acrylamide, respectively, but only 20% by the combination. The present study demonstrates that acrylamide may directly regulate the expression of individual neurofilament subunit genes via distinct mechanisms from NGF, and this effect may contribute to neurofilament accumulation in the distal portion of the axon, a pathognomonic feature of acrylamide neurotoxicity in animals.

1554 POTENTIATION OF PARA-AMINOPHENOL CYTOTOXICITY BY COINCUBATION OF RAT RENAL TUBULES WITH HEPATIC S9 FRACTION

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Para-aminophenol (PAP) nephrotoxicity *in vivo* is characterized by decreases in renal oxygen consumption, adenine nucleotide contents, TEA and PAH uptake within 2–4 h following ip administration (300 mg/kg). In contrast, PAP cytotoxicity was characterized by decreases in oxygen consumption, adenine nucleotide contents and TEA uptake only after incubation of renal tubules with 1 mM PAP for 4 h. Based on the hypothesis that PAP nephrotoxicity involves sequential metabolism by liver and kidney, in present study, we assessed the effects of hepatic and renal metabolism of PAP on cytotoxicity *in vitro*. Renal tubules were prepared by collagenase digestion and incubated with 0.5 mM PAP in the presence or absence of hepatic slices (100 mg) or hepatic S9 fraction (10 mg/ml) with or without 1 mM glutathione (GSH). Incubation of renal tubules with 0.5 mM PAP in the presence or absence of 1 mM GSH failed to produce cytotoxicity within 4 h. Coincubation of renal tubules with liver slices (100 mg) or hepatic S9 fraction (10 mg/ml) did not produce cytotoxicity in tubules incubated with 0.5 mM PAP. However, coincubation of tubules with 1 mM GSH and hepatic S9 fraction (10 mg/ml) produced cytotoxicity in tubules incubated with 0.5 mM PAP at 4 h. The result indicates that 1) PAP (0.5 mM) does not directly produce cytotoxicity in tubules, 2) PAP cytotoxicity is not due to enzymatic metabolism alone, 3) PAP cytotoxicity is not due to autoxidation and spontaneous GSH conjugation, and 4) only hepatic metabolism as well as GSH conjugation, presumably of reactive intermediates derived from hepatic and/or renal metabolism of PAP, potentially mediates PAP cytotoxicity.

1555 ANOXIA INDUCES AN INCREASE IN POLYMERIZED ACTIN IN RABBIT PROXIMAL CONVOLUTED TUBULES

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In cultured renal and endothelial cells, anoxia causes an increase in the fraction of actin that is in the polymerized form. In this communication, we initiated a study of the various actin pools present in proximal convoluted tubules during normoxia and anoxia. Actin was separated into a Triton X100 (TX) insoluble and TX soluble fraction. Immunoblots and subsequent densitometry revealed that actin in the TX insoluble fraction increased 117% after 30 min and 133% after 60 min of anoxia when compared to normoxic values. The TX soluble fraction was then subjected to a high speed centrifugation (105,000g) for 1 hr and actin was measured in the high speed pellet. The high speed pellet may represent a labile pool of polymerized actin. There was an increase in actin of 135% after 30 min and 185% after 60 min of anoxia when compared to normoxic values. We are currently investigating whether all the TX soluble actin is in the G-form and whether actin in the TX insoluble fraction and high-speed pellet is F-actin. The association of actin-binding proteins with actin in these different fractions is also being determined (Supported by NIEHS training grant ESO7031.)

1556 EFFECTS OF CHLORAMPHENICOL, DEHYDROEPIANDROSTERONE (DHEA) AND 2,6-DICHLORO-4-NITROPHENOL (DCNP) ON N-(3,5-DICHLOROPHENYL)-2-HYDROXYSUCCINIMIDE (NDHS) NEPHROTOXICITY

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Previous studies have shown that the agricultural fungicide N-(3,5-dichlorophenyl)succinimide (NDPS) induces nephrotoxicity via metabolites formed following oxidation of the succinimide ring. One such metabolite, NDHS, is four times more potent than NDPS as a nephrotoxicant in Fischer 344 rats. Preliminary studies also suggested that conjugates of NDPS metabolites might contribute to NDPS nephrotoxicity. In this study, male Fischer 344 rats (4/group) were pretreated with inhibitors of sulfation and/or glucuronidation (chloramphenicol, 300 mg/kg, i.p., 15 min.; DHEA 0.5 mmol/kg, i.p., 1 hr; or DCNP, 0.04 mmol/kg, i.p., 45 min.) before a nephrotoxicant dose of NDHS (0.1 mmol/kg, i.p.) or NDHS vehicle. Renal function was then monitored for 48 hr. Pretreatment with DCNP, an inhibitor of phenolic sulfation, only mildly attenuated NDHS nephrotoxicity. Pretreatment with DHEA, an inhibitor of secondary alcohol sulfation, or chloramphenicol, an inhibitor of glucuronidation and sulfation, markedly diminished NDHS-induced renal effects. These results support the hypothesis that conjugation of nephrotoxicant NDPS metabolites at nonphenolic sites is a mechanism for bioactivation, although the exact nature of the nephrotoxicant conjugate(s) remains to be determined. This work was supported by NIH grant DK31210.

1557 AMPHOTERICIN B (AMPB) RENAL INJURY IN RATS AND PROTEIN THIOL STATUS

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The mechanisms responsible for AMPB-induced renal injury are not known.

Because oxidant mechanisms may contribute to AMPB nephrotoxicity, we studied the effects of AMPB in female Fischer-344 (F344) rats. We have observed a slightly greater sensitivity to AMPB renal injury in F344 rats than in Sprague-Dawley rats. Female F344 rats given single doses of 20 mg/kg of AMPB, i.p., developed renal injury evidenced by elevated plasma BUN and creatinine concentrations (59.7 ± 0.6 and 1.03 ± 0.05 mg/dl, respectively; vehicle-treated controls, 13.8 ± 0.7 and 0.46 ± 0.03) that peaked by 48 h, declining to baseline values by day 6 post dose. Evidence of tubular injury and progress through initial stages of tissue repair were also observed by histology. Plasma and tissue levels of AMPB were measured by HPLC. Plasma concentrations showed an initial decline, with a half-life of about 20 h, through day 4, but no further decrease in AMPB concentrations was observed through day 7. The concentrations of AMPB in renal and hepatic tissues were not closely correlated with plasma levels. Interestingly, plasma ALT activities did not increase, despite the 10-fold greater concentrations of AMPB in liver than in kidney. Further, biochemical and histological evidence of recovery of renal injury was observed, even in animals with relatively high concentrations of AMPB. The redox status of renal proteins was assessed by fluorescence labeling with the thiol-specific reagent monobromobimane, followed by separation by SDS-PAGE. No substantive changes in total or in any individual protein thiol was observed, even in animals that showed marked renal damage. Conclusions: 1) Female F344 rats offer a useful model of AMPB-induced renal injury *in vivo*; 2) Recovery of renal function is observed despite the continued presence of AMPB; 3) Protein thiol status is not substantively altered by nephrotoxic doses of AMPB.

1558 PROTEIN DEPHOSPHORYLATION IN RENAL PROXIMAL TUBULAR (RPT) INJURY: EFFECTS OF GLYCINE, STRYCHNINE AND CALYCULIN A

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Glycine and strychnine protect RPT from a variety of toxicants and work in the late phase of cellular injury (TAP 125:192, 1994). Since inhibition of protein phosphatases decreases antimycin A-induced RPT cellular death (Toxicologist 15:296 1995), we tested the hypothesis that the cytoprotective effects of glycine and strychnine are due to their ability to inhibit protein dephosphorylation. Rabbit RPT suspensions were preincubated with $^{32}PO_4$ for 120 min to label intracellular phosphoproteins. Antimycin A (1 μM, mitochondrial inhibitor) and/or cytoprotective concentrations of glycine (2 mM), strychnine (1 mM) and calyculin A (100 nM, protein phosphatase 1 and 2A inhibitor), or diluent were then added. Samples were removed at various times and ^{32}P-protein content determined in perchloric acid precipitates. $^{32}PO_4$-protein content in control RPT increased over the 180 min exposure period. Antimycin A reduced ^{32}P-protein content 28 ± 5 and 34 ± 5% after 30 and 60 min of exposure, respectively. Glycine nor strychnine had any effect on antimycin A-induced protein dephosphorylation. In contrast, calyculin A decreased antimycin A-induced protein dephosphorylation 19 ± 3 and 26 ± 2% after 30 and 60 min, respectively. Using LDH release as a marker of cell death, glycine, strychnine and calyculin A decreased antimycin A-induced RPT cell death. These results show that 1) proteins are dephosphorylated in the absence of ATP, 2) glycine and strychnine are cytoprotective but do not have a significant effect on the protein dephosphorylation, and 3) the cytoprotective effect of calyculin A is associated with its ability to inhibit protein dephosphorylation.

1559 METHYL tert-BUTYL ETHER (MTBE)-INDUCED PROTEIN DROPLET NEPHROPATHY AND CELL PROLIFERATION IN MALE F-344 RATS

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MTBE, an oxygenated fuel additive utilized to decrease carbon monoxide emissions, induces renal tumors in male, but not female, rats. A number of chemicals that cause male rat-specific renal tumors also induce cell proliferation (CP) and a protein droplet nephropathy (PDN). The objective of the present study was to determine if MTBE induces PDN and CP in F-344 rats. Male and female F-344 rats were exposed to MTBE vapors at 0, 400, 1500, or 3000 ppm for 6 hours/day for 10 consecutive days. Three days prior to euthanasia, rats were implanted with an osmotic pump for delivery of 5-bromo-2-deoxyuridine (BrdU). Twenty-four hours following the final exposure to MTBE, rat kidneys were perfused and collected. Significant proximal tubular epithelial cell necrosis was observed in hematoxylin-eosin (H&E) stained sections in male rats exposed to 1500 and 3000 ppm MTBE. The size and intensity of protein droplets within the proximal tubules were also significantly increased at 1500 and 3000 ppm MTBE in male rat kidney stained with Mallory's Heidenhain (MH), a nonspecific protein stain. Immunohistochemical staining of male rat kidney for BrdU revealed significantly greater labeling indices within the proximal tubule in all treated groups as compared to controls. No significant differences were observed in MTBE-treated female rats for cortical lesions as evaluated on H&E sections, BrdU immunohistochemistry, or MH staining in the proximal tubules. MTBE-induced PDN and CP, observed only in male rats, may be associated with male rat-specific renal tumor formation. (Sponsored by the Oxygenated Fuels Association.)

1560 CYCLOSPORINE A INDUCED DECREASE OF THE CALCIUM BINDING PROTEIN CALBINDIN-D 28K IN RAT KIDNEY

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Cyclosporine A (CsA) is an immunosuppressive polypeptide used to prevent allograft rejection. Despite the widespread clinical application of CsA its precise mechanism of action and its side effects still remain incompletely understood. There exists a large body of evidence that disturbance of calcium homeostasis is a critical step in the cascade of cellular and molecular events induced by the drug. As recently shown in our laboratory by two-dimensional protein gel electrophoresis (2-DE) analysis of kidney homogenates, CsA induced abundance changes in several kidney proteins. Particularly one kidney protein was shown to be strongly down-regulated by the drug. Now we report the identification of the strongly decreased kidney protein as calbindin-D 28K, a vitamin D-dependent calcium-binding protein, associated with calcium handling by cells. The assignment of the down-regulated protein spot is based on its internal amino acid sequence analysis and its specific reaction with a monoclonal antibody raised against calbindin-D 28K. In kidney homogenates of male Wistar rats treated with 50 mg/kg/d CsA for up to 28 days, calbindin levels were measured by ELISA and were shown to be continuously decreased with prolonged CsA treatment. To our knowledge this is the first report which describes the effect of CsA on kidney calbindin-D 28K protein levels. Further studies are needed to elucidate whether the CsA-mediated down-regulation of the calcium binding protein calbindin-D 28K may be a critical factor for the renal side effects induced by this drug.

1561 NEUROSTEROIDS ARE CYTOPROTECTIVE IN RENAL PROXIMAL TUBULE (RPT) CELLULAR INJURY

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We have previously shown that the neuronal $GABA_A$ receptor agonist muscimol and the antagonist bicuculline are cytoprotective to RPT exposed to the mitochondrial inhibitor antimycin A. Additional studies demonstrated that increased Cl^- influx occurs in the late phase of cellular injury produced by various toxicants, and that antimycin A-induced Cl^- influx in RPT is not blocked by specific inhibitors of known renal Cl^- transport. This study continued the investigation of the novel cytoprotective effects of neuronal $GABA_A$ receptor modulators using the neurosteroids allopregnanolone and 17α-OH-allopregnanolone. Rabbit RPT suspensions were exposed to antimycin A in the presence and absence of each neurosteroid, and cell death was quantified after 60 min by the release of LDH activity. 17α-OH-allopregnanolone decreased cell death by 66%, 82%, and 84% at 1, 10 and 100 nM, respectively. Allopregnanolone decreased cell death by 84% at 1 nM with no significant increase in cytoprotection at higher concentrations. Immunoblot experiments using mAbs bd-17 and bd-28 (recognize β_2-and β_3-, and α_1-$GABA_A$ receptor subunits, respectively) revealed an immunoreactive protein band corresponding to the β_3-subunit but not the β_2- or α_1-subunits on a western blot of immunoprecipitated RPT homogenates. Furthermore, immunofluorescent microscopy localized an immunoreactive protein corresponding to the β_3-subunit to the basolateral portion of RPT. In conclusion, we have identified a new class of potent cytoprotectants in RPT that are neuronal $GABA_A$ receptor modulators and showed that RPT contain a β_3-like subunit of the $GABA_A$ receptor located on the basolateral membrane. We suggest that the cytoprotective effects of neurosteroids are through modulation of a Cl^--coupled renal $GABA_A$ receptor that is distinct from but related to its neuronal counterpart.

1562 Gd-DCTPA: A NOVEL URINARY CALCULUS-FORMING AGENT IN MALE RATS

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Dicyclohexenetriaminepentaacetic acid is used to chelate gadolinium (Gd-DCTPA). Single intravenous injection of the chelate (100 umol Gd/kg) in male Sprague-Dawley rats resulted in bilateral hydronephrosis and formation of calculus in the urinary bladder (urolithiasis) within 30 days in 1 of 3 rats. In a pharmacokinetic/biodistribution study, 10 groups (n = 4/group) of rats were given ^{153}Gd-DCTPA (100 umol Gd/kg, ~30 uCi/kg) and euthanized at various timepoints after dosing up to 30 days. ^{153}Gd-DCTPA was rapidly removed from blood, the plasma $T_{1/2}\beta$ was 0.4 hr; the mean residence time was 0.7 hr, ~53% of the injected dose was excreted in urine in 24 hours, and there was minimal organ retention of ^{153}Gd after 24 hr. However, 4 of 4 rats necropsied at 30 days showed evidence of hydronephrosis and urolithiasis (9 to 37 mg calculus, struvite). In a 4-week renal function study, rats given Gd-DCTPA i.v. showed a modest transient phosphaturia within 4 hours of dosing; renal excretion of Ca, Mg, Na, K, NH_4, Cl, urea and Cr unremarkable. Again, 2 of 4 rats exhibited hydronephrosis and urolithiasis (23 & 62 mg calculus, struvite). Histopathology showed mineralized deposits in proximal, distal, and collecting tubules. We conclude that single administrations of Gd-DCTPA are associated with urolithiasis and secondary hydronephrosis in male rats. Gd-DCTPA is rapidly eliminated by the rat kidney and may be associated with a transient phosphaturia sufficient to seed precipitation of magnesium-ammonium-phosphate (struvite) in the renal tubular system and urinary bladder.

1563 *IN VITRO* UPTAKE OF METHYL *t*-BUTYL ETHER (MTBE) IN MALE AND FEMALE RAT KIDNEY HOMOGENATE: SOLUBILITY AND PROTEIN INTERACTIONS

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The fuel additive, MTBE, causes renal tumors following chronic exposure in only male rats. In the process of measuring chemical-specific parameters for the development of a dosimetry model of MTBE it was observed that MTBE is 5.5 times more soluble in male F-344 rat kidney than in female kidney, and 4.5 times more soluble in male rat kidney than in liver. The hypothesis was that MTBE interacts reversibly with male rat kidney proteins, leading to a greater uptake of MTBE in this tissue *in vitro*. Vial equilibration was used to compare partition coefficients for MTBE in tissue homogenates. Samples of kidney and liver homogenates were heat-treated prior to incubation with MTBE to diminish possible metabolism and protein binding. Heat treatment of homogenates led to a decrease in the uptake of MTBE in male, but not female, rat kidney and did not affect the uptake of MTBE into the liver. These results imply that metabolism does not play a role in the uptake of MTBE in male rat tissues under these conditions. Heated and non-heated kidney homogenates from male and female rats were incubated with [^{14}C]MTBE and eluted on a PD-10 column to collect the total protein fraction. The percentage of [^{14}C]MTBE that eluted with the total protein fraction was less in the heat-pretreated homogenates when compared to the non-heated samples (44% vs. 61%, respectively). Less than 8% of the [^{14}C]MTBE co-eluted with the total protein fraction from female samples. Although [^{14}C]MTBE co-eluted with the protein fraction from male and not female kidneys, this interaction was easily reversed by dialysis, suggesting weak or non-specific [^{14}C]]MTBE-protein binding. (Sponsored by the Oxygenated Fuels Association.)

1564 COMPARATIVE DISPOSITION OF N-(3,5-DICHLOROPHENYL)-SUCCINIMIDE AND N-(3,5-DIFLUOROPHENYL)SUCCINIMIDE IN F344 RATS

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Disposition of the nephrotoxicant, N-(3,5-dichlorophenyl)succinimide (NDPS) and N-(3,5-difluorophenyl)succinimide (DFPS), a non-toxic analogue, was compared. Male Fischer 344 rats were dosed with 0.2 or 0.6 mmol/kg ^{14}C-NDPS or ^{14}C-DFPS (10 μCi; *ip* in corn oil). Plasma concentrations were determined from blood samples obtained through a carotid artery cannula. Urine samples were counted and deproteinized for HPLC analysis. Rats were sacrificed at 3 hrs (DFPS) or 6 hrs (NDPS) and liquid scintillation counting was used to determine tissue radiolabel content and covalent binding. Plasma levels of radioactivity from ^{14}C-NDPS were significantly higher and peaked much later than those from ^{14}C-DFPS. Six hours after dosing ^{14}C-NDPS was 40 % eliminated in the urine whereas ^{14}C-DFPS was > 87 % excreted. By 48 hrs, 33 % of the ^{14}C-NDPS dose was unaccounted for in urine, compared to only 5 % for ^{14}C-DFPS. Similar metabolic profiles were found in urine for both compounds. At the time point where each compound was approximately 50 % eliminated in urine, ^{14}C-NDPS tissue levels were greater than ^{14}C-DFPS levels, especially in the kidney (4-fold) and serum (6-fold). ^{14}C-NDPS (0.2 mmol/kg) covalently bound to proteins to a greater extent than ^{14}C-DFPS in all tissue examined. Most notable was a 5-fold higher binding to kidney and >8-fold binding to serum. These differences were maintained at a 0.6 mmol/kg dose. In summary, the toxic analogue, NDPS, reaches higher serum and tissue levels, covalently binds to a greater extent, and is eliminated much slower than DFPS. Differences in the lipid solubility of NDPS-derived metabolites and DFPS metabolites may help explain these results. The overall greater tissue exposure of NDPS and its metabolites may contribute to NDPS-induced nephrotoxicity. Supported by USPHS grant ES05189.

1565 EXPERIMENTAL NEPHROTOXICITY OF LY264826 (Chloroorienticin A) IN MALE FISCHER 344 RATS: A MODEL GLYCOPEPTIDE ANTIBIOTIC

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Glycopeptide antibiotics have been a clinically important group of anti-infectives since the introduction of vancomycin in 1958. Administration of glycopeptide antibiotics has long been associated with nephrotoxicity in experimental animal models. In recent years, the appearance of antibiotic resistant Gram-positive pathogens has triggered a dramatic increase in their clinical use and a subsequent search for novel glycopeptide compounds. LY264826 is a glycopeptide molecule with a chemical structure and mode of action similar to that of vancomycin. We have used LY264826 as a model glycopeptide molecule with which to investigate and characterize the nephrotoxicity associated with this class of antibiotics. Initially, we administered single i.v. doses of 100, 250 and 500 mg/kg followed by time course analysis from 24 to 96 hours. Functional decrement and histopathological changes were seen in all treatment groups. BUN was increased in the low-, mid-, and high- dose groups at 24 hours by 58%, 232% and 354%, respectively. Changes in additional functional parameters, such as creatinine clearance and fractional excretion of sodium, were also evident. In a time course study of 14 daily i.v. administrations of LY264826, a biphasic response was observed. This response involved initial functional changes which peaked at 24 hours, recovery to control level by day 4, followed by a secondary phase of renal dysfunction evident through day 14.

1566 MODULATION OF MERCURIC CHLORIDE INDUCED KIDNEY INJURY BY TWO RECOMBINANT HUMAN EPIDERMAL GROWTH FACTORS

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Effects of two biologically active epidermal growth factors, EGF_{1-48} and EGF_{1-53}, were investigated in a model of renal tubular injury. Rats received 5 mg/kg mercuric chloride ($HgCl_2$) SC followed by a single SC dose of saline, 100 μg/kg EGF_{1-48}, or 100 μg/kg EGF_{1-53}. $HgCl_2$ was lethal to greater than 50% of the rats whether or not EGF was administered. Animals given either EGF_{1-48} or EGF_{1-53} had more PCNA-labeled tubular cells from Days 1–5 than animals receiving $HgCl_2$ alone. Tubular regeneration also was seen earlier in EGF groups. Through Day 4, distal tubules contained more PCNA-positive cells and more frequent mitoses than proximal tubules, most prominently in EGF groups. Multiple layers of tubular epithelial cells appeared in EGF animals, with the greatest effect seen with EGF_{1-53}. In all groups, irrespective of EGF treatment, creatinine and BUN were increased greater than 10- and 16-fold, respectively, at Days 3–4. By Day 8, kidneys recovered with only small areas of focal damage. These results show that both growth factors stimulate tubular epithelial cell proliferation, and EGF_{1-53} is more potent than EGF_{1-48} for this effect in the rat. However, while both EGF_{1-48} and EGF_{1-53} accelerated tubular regeneration and repair, no concurrent improvement was seen in renal function or survival.

1567 BILIARY CANNULATION OR LIGATION REDUCES RENAL ACCUMULATION OF INORGANIC MERCURY

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In the present study, we evaluated the effects of biliary cannulation and ligation on the renal disposition of mercury in rats administered (i.v.) a 0.5 μmol/kg nontoxic dose of mercuric chloride ($HgCl_2$) containing radioactive inorganic mercury $^{203}Hg^{++}$. Biliary cannulation caused the amount of mercury in the blood to increase significantly (about 40 and 50%, respectively) and caused the renal accumulation of mercury to be decreased significantly (by about 21 and 33%, respectively) at both 1 and 3 h after the injection of $HgCl_2$. The decreased renal accumulation of mercury was due to diminished uptake of mercury in the cortex and outer stripe of the outer medulla. Hepatic accumulation of mercury was not affected significantly by biliary cannulation, and the excretion of mercury in the bile was very low. The amount of mercury excreted in the bile (less than 0.25% of the dose) could account for no more than about 4% of the quantity of mercury not taken up by the total renal mass. Renal accumulation of mercury, specifically in the cortex and outer stripe of the outer medulla, was also greatly reduced by biliary ligation, when evaluated 24 hours after the injection of $HgCl_2$. Biliary ligation caused an almost fourfold increase in the hepatic content of mercury, and caused the fecal excretion of mercury to be reduced by about 85%. What is interesting is that some fecal excretion occurred despite biliary ligation. This finding would seem to indicate that there is some secretion of mercury along some portion of the intestines. In conclusion, the present findings indicate that the liver plays a role in the renal accumulation of inorganic mercury. This role does not appear to involve a biliary-enteric-hepatic cycling of mercury.

1568 MOLECULAR, BIOCHEMICAL, AND HISTOLOGICAL ALTERATIONS INDUCED BY CISPLATIN ADMINISTRATION IN MICE

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The nephrotoxic potential of cisplatin is well known, yet the molecular events leading to subsequent biochemical and histological changes are only beginning to be explored. To examine dose and time-dependent nature of events, male CD-1 mice were administered single intravenous doses of 0, 4, 7, or 10 mg/kg cisplatin. Four animals from each group were killed 1, 2, 6, 24 or 96 hours postdose. Dose-dependent body weight loss was evident at 96 hr. No statistically significant elevations were seen in BUN and creatinine at 24 or 96 hr (a 2-fold increase in BUN was noted in 2 of 4 high dose animals at 96 hr). Renal lesions were not evident at 4 mg/kg; minimal degeneration and/or necrosis of tubular epithelial cells was seen in one animal at 7 mg/kg and 3 animals at 10 mg/kg. To measure alterations in gene expression, samples of kidney, liver, spleen, and lung were collected at each time point and flash frozen. Total RNA was extracted from each tissue and quantitated. Reverse transcriptase polymerase chain reactions (RT-PCR) were performed on selected samples. PCR products were visualized on ethidium bromide stained gels. Preliminary screens indicate the expression of p53 was unchanged by cisplatin treatment. Additional transcriptional activators (c-fos, c-myc) are being investigated.

1569 IMMUNOHISTOCHEMICAL EVALUATION OF ALPHA$_{2u}$-GLOBULIN IN KIDNEYS OF MALE RATS FED HC ORANGE NO. 1 IN THE DIET FOR 13 WEEKS

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Dietary administration of HC Orange No. 1, a semi-permanent (direct) hair dye, to Sprague-Dawley rats at levels up to 1.25% for 13–27 weeks produced an increased incidence and severity of hyaline droplets in male rat kidneys. In order to establish whether this was due to an increased accumulation of alpha$_{2u}$-globulin, an effect considered not relevant for human risk assessment, paraffin sections of kidney from 10 male rats receiving 0.125%, 0.4% and 1.25% HC Orange No. 1 and from 10 high-dose female rats were stained immunohistochemically using a monoclonal antibody against alpha $_{2u}$-globulin. Kidney slides from rats administered d-limonene, 150 mg/kg/day for 4 days, were included as a positive control. Direct comparisons of hematoxylin and eosin (H&E) and immunochemistry-stained sections were performed. A clear relationship was present for alpha $_{2u}$-globulin immunostaining and the size and number of brightly colored eosinophilic droplets in treated male but not female rats. Thus, the increase in hyaline droplets was associated with accumulation of alpha $_{2u}$-globulin. H & E slides also confirmed increased numbers of regenerative tubules in treated male rats. We conclude that HC Orange1 induces alpha 2u-globulin nephropathy but this endpoint should not be used for human risk assessment or for setting a NOAEL.

1570 BOSENTAN (BST), A MIXED ENDOTHELIN ANTAGONIST, PROTECTS AGAINST HEXACHLORO-1,3-BUTADIENE(HCBD) NEPHROTOXICITY

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Endothelin is a 21 amino acid peptide produced by many different cell types. Its' effects on the kidney are mediated by two receptor subtypes and mimic those seen in acute renal failure. HCBD is a by-product of the synthesis of chlorine-containing solvents that causes an intense acute renal failure. In this study, the role of endothelin in HCBD-induced acute renal failure was examined using the mixed endothelin receptor antagonist BST. Male Sprague-Dawley rats were pretreated with BST (10mg/kg) or water followed by HCBD (100mg/kg) or oil. BST pretreatment blunted the HCBD-induced increase in BUN (at 48 hrs.-44 ± 6mg/dL vs. 33 ± 3mg/dL; at 72 hrs.-47 ± 9mg/dL vs. 27 ± 5mg/dL; at 96 hrs.-64 ± 15mg/dL vs. 44 ± 14mg/dL). HCBD increased glucose excretion in the water pretreated rats at 24 and 48 hours (95 ± 26mg vs. 5 ± 2mg and 75 ± 26mg vs. 2 ± 3mg). BST pretreatment blunted this effect at both time points (56 ± 21mg vs. 5 ± 2mg and 54 ± 29 vs. 2 ± 1mg). Proteinuria was evident in the water/HCBD treated rats at all four time points (24 hrs.-171 ± .1mg vs. 53 ± 3mg.; 48 hrs.-149 ± 4mg vs. 84 ± 4mg; 72 hrs.-266 ± 4mg vs. 56 ± 1; 96 hrs.-179 ± 6mg vs. 48 ± 1mg). At the latter two time points, BST pretreatment diminished the increase in protein excretion induced by HCBD (72 hrs.-174 ± 9mg vs. 69 ± 4m; 96 hrs.-110 ± 8mg vs. 83 ± 4mg). Blocking endothelin receptors with BST offers partial protection from HCBD-induced acute renal failure. These results support the hypothesis that endothelin mediates HCBD-induced acute renal failure. (This research was supported by NIH Training Grant GM 07039–20 and 1R25RR10223 and Univ. Health Assoc.)

1571 EFFECT OF ASCORBIC ACID AND ACIVICIN ON 4-AMINO-2, 6-DICHLOROPHENOL (ADCP)-INDUCED NEPHROTOXICITY IN THE FISCHER 344 RAT

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A halogenated derivative of 4-aminophenol, 4-amino-2, 6-dichlorophenol (ADCP), is a potent nephrotoxicant and a hepatotoxicant in vivo and in vitro. ADCP is more toxic to the kidney than to the liver, inducing prominent renal corticomedullary necrosis. Although the mechanism of ADCP nephrotoxicity has not been studied, ADCP can undergo oxidation to 4-aminophenoxy radical or 1,4-benzoquinoneimine which can covalently bind to cellular macromolecules. This toxic process might be mediated by glutathione conjugate of ADCP, as suggested for the mechanism of 4-aminophenol nephrotoxicity. In this study, the effect of ascorbic acid, an antioxidant, and acivicin (AT-125), an inhibitor of γ-glutamyltransferase, on the nephrotoxicity induced by ADCP was examined. Male Fischer 344 rats (4/group) were intraperitoneally (i.p.) administered ADCP (0.38 mmol/kg) only, co-administered ADCP (0.38 mmol/kg) with ascorbic acid (1.14 mmol/kg) or pretreated with acivicin (10 mg/kg) 1 h prior to ADCP (0.38 mmol/kg), and renal and hepatic function was monitored for 48 h. Administration of ascorbic acid markedly reduced ADCP-induced nephrotoxicity, with only slight glucosuria and hematuria observed, compared to the marked changes in renal functional parameters induced by ADCP. Pretreatment with acivicin also resulted in some protection against the nephrotoxicity induced by ADCP. The results indicate that oxidation of ADCP to form a toxic metabolite may play an important role in ADCP nephrotoxicity and that a glutathione conjugate of ADCP may be partly responsible for the toxic effects of ADCP. Supported by NIH grant ES04954.

1572 COMPARISON OF CEPHALORIDINE *IN VITRO* RENAL TOXICITY BETWEEN NORMOGLYCEMIC AND DIABETIC RATS

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Cephaloridine nephrotoxicity was reduced in a diabetic rat model. The follow-

ing studies investigated whether the reduction in toxicity would be apparent following in vitro exposure to cephaloridine. Diabetes was induced in male Fischer 344 rats by intraperitoneal injection with 35 mg/kg STZ. Renal tissue was collected under diethyl ether anesthesia 7–10 days after STZ injection. Renal slices were incubated for 120 min with 0–5 mM cephaloridine. Cytotoxicity was monitored by measurement of lactate dehydrogenase (LDH) release in normoglycemic and diabetic tissue. Pyruvate directed gluconeogenesis was also monitored following a 90 min pre-incubation with 0–5 mM cephaloridine. Renal cortical slice accumulation was lower in the diabetic relative to the normoglycemic group at concentrations $\leq$ 2 mM. Renal cortical accumulation was comparable between normoglycemic and diabetic tissue for cephaloridine concentrations > 2 mM. LDH leakage was increased ($p < 0.05$) in the normoglycemic tissue following exposure to 4–5 mM cephaloridine. LDH leakage was not elevated in the diabetic group incubated with 0–5 mM cephaloridine. Gluconeogenesis was decreased in the normoglycemic and diabetic tissue following exposure to 2–5 mM. These results would support the hypothesis that the diabetic state was associated with cellular differences that modify cephaloridine toxicity. (Supported by NIH DK45099).

1573 EFFECTS OF BENZODIAZEPINE (BZD) AGONISTS AND ANTAGONISTS ON LINDANE-INDUCED TOXICITY IN RENAL CORTICAL SLICES

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Lindane (LIN), an organochlorine insecticide, functions as a GABA/BZD antagonist in the central nervous system. Peripheral GABA/BZD-like receptors have been localized to the distal nephron of the kidney. We have previously shown that LIN incubation with renal cortical slices produced an increased kallikrein release, suggesting a distal tubular effect. This effect was ameliorated by coincubation with GABA. The current study was designed to further examine the interaction between LIN and BZD agonists and antagonists *in vitro*. Renal cortical slices from Sprague-Dawley rats were incubated with LIN($1x10^{-6}$M) alone; LIN plus PK-11195, a peripheral BZD receptor antagonist ($5x10^{-6}$, $1x10^{-6}$, $5x10^{-7}$M); LIN plus flurazepam HCL, a BZD receptor agonist ($5x10^{-6}$, $1x10^{-6}$, $5x10^{-7}$M) for 2 hours. The incubation medium was analyzed for kallikrein release. PK-11195 produced a concentration-dependent increase in kallikrein release (control-41.82 ± 6.57, hi-95.47 ± .82, med-80.87 ± 1.4, low-72.75 ± .42 U/mg tissue). LIN plus PK-11195 did not produce an additive kallikrein release. Flurazepam ($5x10^{-6}$, $1x10^{-6}$M) significantly reduced LIN-induced kallikrein release (hi-50.72 ± 9.2, med-52.72 ± 5.67, LIN-75.36 ± 1.21 U/mg tissue, respectively). These data suggest a role for a BZD/GABA receptor-mediated interaction for LIN-induced toxicity in renal cortical slices.

1574 LIVER/KIDNEY SLICE CO-INCUBATION: A MODEL FOR INTERACTIVE ORGAN TOXICITY

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Chlorotrifluoroethylene (CTFE), a monomeric precursor used in the plastics industry, has been shown to produce kidney toxicity in several species. This toxicity is due to complex, multistep renal secondary metabolism of the hepatic primary metabolite of CTFE, S-(2-chloro-1,1,2-trifluoroethyl) glutathione (CTFE-SG), to reactive sulfur species. We have investigated the suitability of a co-incubation system combining liver and kidney slices in roller culture and allowing for exposure to a volatile or gaseous toxicant as an in vitro model for this complex interaction involving several organ-specific metabolic steps and terminating in the production of a reactive molecular species. Chemical synthesis of the CTFE-SG according to Dohn et al. allowed us to elucidate the toxicity dose response relationship in rat and rabbit kidney slices as well as HPLC analysis of the kinetics of its appearance from rat and rabbit liver slices. Kidney slices representing all regions of the organ and positional slices rich in the areas of specific toxicity which had been observed in vivo, as well as endpoints comprising a regio-selective (PAH transport) and a highly sensitive one (ATP) were used. Funded in part by NIEHS 05790 (AJG, KB) a fellowship from NIEHS training grant ES 07091 (SJH), and by core facilities of the Southwest Environmental Health Sciences Center ES 06694.

1575 EFFECT OF CIMETIDINE ON RENAL VANCOMYCIN (VAN) ACCUMULATION AND TOXICITY

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VAN is a nephrotoxic cationic glycopeptide antibiotic used in the treatment of serious gram positive infections. We have demonstrated previously that the extent of VAN accumulation in renal tissue is correlated with the degree of observed nephrotoxicity. The mechanisms for renal handling of VAN are still not clear. Sokol (JPET 259: 1283-1287, 1991) showed that VAN is transported in rabbit renal basolateral membrane vesicles via the organic cation transporter. The purpose of the present study was to determine the effect of cimetidine (CMT), an inhibitor of renal cationic secretory transport, on VAN accumulation and subsequent toxicity in Sprague-Dawley rats. VAN (450 μmole/kg) was administered i.p. Rats were pretreated with cimetidine (450 μmole/kg or 900 μmole/kg) s.c. 30 minutes prior to VAN administration. Serum and kidney VAN concentrations were determined by HPLC 3 hrs post dosing. Kidney weights and blood urea nitrogen (BUN) determinations were also evaluated. Compared to VAN alone, CMT (900 μmole/kg) pretreatment resulted in a significantly lower kidney weight (.848 ± .037 vs .653 ± .022 gm, respectively, $p \leq .05$), BUN values (40.65 ± 2.75 vs 19.27 ± .636 mg%, respectively, $p \leq .01$), and kidney VAN concentrations (8822 ± 1208 vs 161 ± 34.20 μgm/gm tissue, $p \leq .01$). These data suggest that VAN may enter rat renal tissue via an organic cation transport mechanism and that CMT can alter VAN disposition. (Supported by NIGMS R29-GM47549).

1576 INHIBITION OF NA^+/GLUCOSE COTRANSPORT BY CISPLATIN IN BRUSH-BORDER MEMBRANE VESICLES: ROLE OF SULFHYDRYL GROUPS

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Cisplatin (cDDP) has been shown to inhibit the Na^+-coupled uptake of methyl-α-D-glucopyranoside (MGP) at non-cytotoxic concentrations in renal proximal tubular cells in primary culture. The Na^+/glucose cotransporter possesses essential sulfhydryl (SH) groups for its activity and platinum exhibits a high affinity for the SH groups. To study the direct interactions of cDDP with the Na^+-coupled uptake of MGP, rabbit renal brush-border membrane vesicles (BBMV) were incubated (4 hours) with cDDP at concentrations ranging from 0.04 to 2 mM, in chloride-free medium. The inhibition of Na^+-coupled uptake of MGP by cDDP (i) increased in a concentration-dependent manner, (ii) was mainly due to a decrease in V_{max} (2 fold) while the K_m value was only slightly increased (20%), (iii) was in keeping with a decrease in protein-bound SH groups and the binding of platinum to BBMV. In BBMV preincubated with cDDP, diethyldithiocarbamate (i) partially restored the activity of Na^+-coupled uptake of MGP (ii) decreased in part the amount of platinum bound to BBMV, but (iii) did not restore the protein-bound SH groups. We conclude that the direct interaction of cDDP with the protein-bound SH groups of the BBMV is involved in the inhibition of the Na^+-coupled uptake of MGP. Our results also suggest that the inhibition of the Na^+-coupled uptake of MGP by cDDP in BBMV is mediated by an interaction with other groups, possibly the $-SCH_3$ group of methionine.

1577 CALPAIN ACTIVITY INCREASES IN RENAL PROXIMAL TUBULAR (RPT) CELL DEATH

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While calpain inhibitors decrease toxicant-induced RPT cell death (Can. J. Physiol. & Pharmacol. 72:602, 1994), measurement of calpain activity is difficult due to calpain activation during membrane permeabilization and the lack of specific substrates. The goal of this study was to develop an *in situ* assay of calpain activity in RPT, determine if the mitochondrial inhibitor antimycin A increases calpain activity, and investigate whether the cytoprotectants glycine and strychnine inhibit calpain activity. Preincubation of RPT with inhibitors of serine proteases (0.2 mM dichloroisocoumarin, 0.1 mM TLCK), acid protease (0.2 mM pepstatin), or cysteine protease (0.5 mM leupeptin) for 30 min decreased RPT hydrolysis of the fluorescent "calpain" substrate sucleu-leu-val-try-AMC (0.05 mM) by 11, 18, 3 and 2%. In contrast, 1 mM calpain inhibitor I decreased calpain substrate hydrolysis by 51%. Thus, non-calpain protease inhibitors have minimal effects on calpain substrate

hydrolysis. TLCK was added in all further studies to increase the specificity of calpain substrate hydrolysis. Addition of antimycin A to RPT increased calpain substrate hydrolysis 25% at 60 min. Calpain inhibitor I was cytoprotective to RPT exposed to antimycin A and decreased calpain substrate hydrolysis by 81%. Glycine (2 mM) and strychnine (1 mM) had no effect on calpain substrate hydrolysis in control RPT. However, glycine and strychnine prevented the increase in calpain substrate hydrolysis and were cytoprotective in RPT exposed to antimycin A. These results suggest that calpain activity increases in the late phase of antimycin A-induced RPT cell injury and that calpain inhibitor I, glycine, and strychnine are cytoprotective in part by their ability to inhibit calpain activation.

1578 WIN 22181: SEX-SPECIFIC RENAL ACCUMULATION OF A 20 Kd POLYMER IN RATS

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WIN 22181 is a linear co-polymer of DTPA and 1,6-hexanediamine chelated with gadolinium (~20Kd). In female Sprague-Dawley rats, ^{153}Gd-WIN 22181 (60 umol Gd/kg, i.v.) is rapidly cleared from blood ($T_{1/2}$ = 0.71 hr) and ~30% of the injected dose (ID) is excreted in urine in 24 hr. Radioactivity is retained in renal cortex (RC), increasing gradually from 6%ID (1 hr) to 14%ID (7 d), and decreases to 5%ID (30 d). Tissue relaxivity measurements suggest that retained ^{153}Gd is WIN 22181. Subsequent studies confirm retention of up to 21% ID in RC, peaking at ~ 2 days post-dosing. RC retention is not associated with changes in renal morphology or function (e.g. Na, K, glucose, creatinine, urea excretion) at up to 30 days post-dosing. In male Sprague-Dawley rats, ^{153}Gd-WIN 22181 is also rapidly cleared from blood ($T_{1/2}$ = 0.76 hr) and ~60% ID is excreted in urine in 24 hr. However, in male rats radioactivity in RC is only 1% ID (7 d). We conclude that WIN 22181 is rapidly cleared from the blood by kidney of both sexes. However, once in the renal tubule, WIN 22181 undergoes sex-specific processing, being reabsorbed and sequestered in renal cortical tissue (most likely in the early proximal tubule) of female rats, but not of male rats. This is the first example, of which we are aware, of sex-specific polymer retention in female rats.

1579 ESTABLISHMENT OF A TRANSEPITHELIAL GRADIENT OF ^{14}C-TEA BY LLC-PK$_1$ CELLS GROWN ON FILTERS

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The renal cell line LLC-PK$_1$ has been shown to transport the organic cation tetraethylammonium bromide (TEA). Our studies were designed to compare unidirectional transport of TEA from the apical (A) side with that from the basolateral (B) side of the cell and to describe the time course. ^{14}C-TEA and ^{3}H-mannitol were added to the transport buffer on either side (top chamber, A, or bottom chamber, B) of confluent monolayers, and flux and uptake measured over 10 hr. ^{3}H-mannitol was used to correct both uptake and flux measurements. Cellular uptake of ^{14}C-TEA from the basolateral side increased rapidly to 27.6 ± 0.5 pmol/mg DNA after 1 hr of incubation and the intracellular concentration remained constant until 6 hr, subsequently decreasing to 16.8 ± 2.3 at 10 hr. In contrast, following exposure from A the intracellular accumulation attained 5.9 ± 0.4 pmol/mg DNA after 1 hr, then increased slowly to 13.4 ± 1.1 pmol/mg DNA at 10 hr. Transfilter flux of TEA from chamber A → chamber B was minimal, attaining only 176 ± 40.3 pmol/cm^2 after 10 hr. In the B → A direction, transepithelial flux was 290 ± 12.3 pmol/cm^2 at 1 hr and increased continuously over the course of the experiment, reaching 1770 ± 35 pmol/cm^2 after 10 hr. Cimetidine (250 μM) inhibited B → A flux after 4 hr but had no effect on cellular uptake at this time point. As a result of B→ A flux of ^{14}C-TEA, the concentration in the apical compartment increased such that after 4 hr of incubation the ratio of [^{14}C-TEA] in the top compartment to that in the bottom was 0.99 ± .03. After 10 hr, the ratio was 2.93 ± .09. These findings confirm the ability of LLC-PK$_1$ cells to transport TEA preferentially from B → A. Additionally, we demonstrate that the flux is linear for at least 10 hr and establish that the cells can create and maintain a transepithelial gradient of TEA.

1580 GLOMERULAR MESANGIAL CELLS ARE PREFERENTIAL TARGETS OF BENZO(A)PYRENE IN THE KIDNEY

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The premise that the kidney is insensitive to benzo(a)pyrene (BaP) toxicity is based on data showing that renal tubular epithelial cells (TECs) are resistant to injury by BaP and related aromatic hydrocarbons. Recent studies in this laboratory have confirmed these findings, but identified glomerular mesangial cells (GMCs) as sensitive targets of BaP toxicity. Sequential exposure of GMCs to BaP (3 μM) alone or in combination with methoxamine (MeoA, 10 μM) for three passages has been shown to induce the acquisition of highly proliferative phenotypes. The current studies were designed to characterize the adaptive response of TECs and GMCs to BaP challenge *in vivo* and *in vitro*. *In vivo* exposure of rats with BaP (10 mg/kg) for 8 weeks was associated with glomerular hypercellularity in the absence of tubular changes. An *in vitro* initiation/promotion strategy using BaP alone or in combination with MeoA did not alter TEC phenotypes, but induced proliferative phenotypes in GMCs. The ability of BaP to modulate phenotypic expression in GMCs was comparable following single and repeated challenges suggesting that genetic changes occur shortly after BaP exposure. These data support the view that GMCs are preferential targets of BaP in the kidney, and provide a mechanistic basis for the incidence of glomerulonephritic disorders in humans exposed to aromatic hydrocarbons. (Supported by NIH grants ES 04917 and ES 00213).

1581 EFFECT OF HEPATIC S9 FRACTION COINCUBATION WITH RENAL CORTICAL SLICES ON N-(3,5-DICHLOROPHENYL)SUCCINIMIDE (NDPS) AND NDPS METABOLITE NEPHROTOXICITY

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NDPS and two of its metabolites, N-(3,5-dichlorophenyl)-2-hydroxysuccinimide (NDHS) and N-(3,5-dichlorophenyl)-2-hydroxysuccinamic acid (2-NDHSA), are nephrotoxicants in vivo, but induce little evidence of nephrotoxicity in vitro. Recent studies have suggested that further hepatic bioactivation may be required before the ultimate nephrotoxicant species is produced from NDHS and/or 2-NDHSA. The purpose of this study was to examine the effects of coincubating hepatic S9 fractions with renal cortical slices from untreated male, Fischer 344 rats in the presence or absence of NDPS, a known or putative NDPS metabolite, or an appropriate vehicle on the nephrotoxic potential of the test compound (final bath concentration of 0.1 or 1.0 mM). Incubations were conducted for 1.5 hr, and renal gluconeogenesis and lactate dehydrogenase (LDH) release were then measured following an additional 0.5 hr incubation in the presence of 10 mM pyruvate. In the absence of hepatic S9 fractions, NDPS (1 mM), but not NDHS or 2-NDHSA, induced a small increase in LDH release, while only 2-NDHSA (0.1 or 1.0 mM) reduced gluconeogenesis. In the presence of hepatic S9 fractions, 2-NDHSA effects on gluconeogenesis were attenuated, while NDHS (1.0 mM) effects on LDH release and gluconeogenesis became significant. Effects induced by other known or putative NDPS metabolites were generally reduced in the presence of the S9 fractions. These results suggest that coincubation of hepatic S9 fractions with renal cortical slices does not provide an adequate in vitro model to investigate NDPS-induced nephrotoxicity. Supported by NIH grant DK31210.

1582 RISK ASSESSMENT AND RISK MANAGEMENT OF WORKER EXPOSURES TO PESTICIDES: THE NEXT GENERATION: INTRODUCTION

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This workshop is designed to inform SOT members of significant new developments in pesticide worker exposure assessment, risk assessment, and risk management. Pesticide risk assessment and risk management are undergoing a dramatic evolution as the regulatory agencies move away from convention NOAEL point estimates of hazard and similar point estimates of exposure towards use of probabilistic models that estimate distributions of hazard and exposure. The first speaker will describe the hazard data that are developed for worker risk assessments and changes in how they are being used in risk assessments. The second speaker will explain the current default assumptions that apply to pesticide worker exposures and risk assessments and ways to improve the measurements and assumptions about exposure. The third speaker will describe the current databases, such as the Pesticide Handlers Exposure Database (PHED) and new models, incorporating Monte Carlo Estimations, that are used to generate estimates of exposure for workers. The fourth speaker will focus on how the US EPA is currently using these exposure and hazard data to make risk assessment and risk management decisions for pesticide workers and the implications of Monte-Carlo-based risk assessments

on the risk management process. The last speaker will describe similar regulatory decisions made by the California EPA.

1583 SPHINGOLIPIDS - THE ENIGMATIC LIPID CLASS: BIOCHEMISTRY, PHYSIOLOGY, AND PATHOPHYSIOLOGY

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The "sphingo" in sphingolipids comes from the fact that in 1884 J.L.W. Thudichum found sphingolipids to be "Sphinx-like" in that they were an enigma. While still an elusive class of lipids, research on complex sphingolipids, free sphingoid bases, and sphingolipid degradation products in signal transduction pathways, and as mediators of cell growth, differentiation, and cell death has been rapidly expanding. Recently, sphingolipid metabolites have been demonstrated to modulate cellular calcium homeostasis and cell proliferation via interaction with specific cellular receptors. Also, disruption of sphingolipid metabolism has been implicated in several animal diseases and human cancer by toxins that are currently the focus of a FDA long term tumor study. This symposium will focus on (i) the role of sphingolipids as important nutrients and contributing factors in disease, (ii) the involvement of disrupted sphingolipid metabolism in animal disease and cellular deregulation associated with exposure to inhibitors of ceramide synthase, (iii) glucosphingolipids as mediators of cell cycle progression and stress responses, and (iv) the role of sphingoid base metabolites and the ceramide cycle in expression of genes regulating cell growth, differentiation, and apoptosis.

1584 NUTRITIONAL SIGNIFICANCE OF SPHINGOLIPIDS IN CANCER AND OTHER DISEASES

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The lipid backbones of sphingolipids (sphinganine, sphingosine, and their 1-phosphates and N-acyl derivatives) are highly bioactive species that affect cell growth, differentiation and programmed cell death. These compounds are released during the digestion of dietary sphingolipids, but little is known about their fate or how they affect gastrointestinal physiology. Recent studies have found that feeding milk sphingomyelin to CF1 mice treated with dimethylhydrazine reduced the appearance of aberrant colonic crypts and the number of colonic adenocarcinomas; but, had no adverse effects on weight gain, nor the health of mice that had not been treated with dimethylhydrazine. Analyses of the sphingolipid composition of normal colon, colon tumors, and colon tumor-derived cell lines have uncovered differences in the ceramide backbones that may be involved in the susceptibility of the colon to neoplasia. These findings establish that sphingolipids should be considered in studies of diet and colon carcinogenesis, both with respect to chemoprevention and, perhaps, chemotherapy. Supported by funds from Dairy Management, Inc. & NCI Grant CA61820.

1585 SPHINGOLIPIDS METABOLITES: MODULATORS OF CELL GROWTH, DIFFERENTIATION AND APOPTOSIS

S Spiegel. *Georgetown University Medical Center, Washington, DC.* Sponsor: R T Riley

Recent evidence suggests that in analogy with a glycerophospholipid cycle, sphingolipid metabolism also generates intermediates which have important biological functions. Branching pathways of sphingolipid metabolism may mediate either mitogenic or apoptotic effects depending on the cell type and the nature of the stimulus. Ceramide has been reported to induce apoptosis and cell arrest, whereas sphingosine and its metabolite, sphingosine-1-phosphate (SPP) generally provide positive mitogenic signals. Several cytokines, including TNF-α, α-interferon, and IL-1 stimulate the activity of sphingomyelinase which catalyzes sphingomyelin degradation and results in the formation of ceramide. The cellular targets mediating the action of ceramide will be discussed. The potent mitogens, PDGF and serum, but not EGF, increased levels of sphingosine and SPP. PDGF and serum also activated cytosolic sphingosine kinase activity. An inhibitor of sphingosine kinase reduced DNA synthesis induced by PDGF and serum but did not abrogate cellular proliferation induced by EGF. Thus elevation of sphingosine and SPP levels and activation of sphingosine kinase may have important biological roles in signal transduction pathways activated by certain growth factors. These metabolites also trigger dual signal transduction pathways of calcium mobilization and activation of phospholipase D, prominent events in the control of cellular proliferation. Sphingosine- and SPP-induced activation of the transcription factor activator protein-1, which is one of the transcription factors activated by the Ras/MKK/MAPK pathway, is the first link between the effects of sphingolipids metabolites on cellular proliferation and gene expression.

1586 EVALUATING THE ROLE OF SPHINGOLIPIDS IN CELL GROWTH AND STRESS RESPONSES THROUGH THE USE OF CEREBROSIDE SYNTHASE INHIBITORS

J A Shayman. *Nephrology Division, Department of Internal Medicine, University of Michigan, Ann Arbor, MI.* Sponsor: R T Riley

Sphingolipids, including ceramide, have been identified as important candidate mediators of cell growth and differentiation phenomena. Establishing a causal role for a particular sphingolipid is often problematic since exogenously administered sphingolipids may not enter their normal cellular compartments and because they may be metabolized to other bioactive lipids. Endogenous cell ceramides may be increased by use of inhibitors of glucosylceramide synthase. Using (R,R) 1-phenyl-2-decanoylamino-3-morpholino-propanol (PDMP) as a parent compound, stereospecific homologues have been created which vary in their ability to change ceramide or block glycolipid synthesis respectively and thus can dissociate cellular changes in ceramide and glycosphingolipids. Studies with these compounds have provided support for a primary role for ceramide in both cell cycle progression and the cellular heat shock response. We have observed that renal growth is particularly sensitive to inhibition of cerebroside metabolism. Preliminary work indicates that ceramide metabolism may mediate the aberrant renal growth response in two common diseases of the kidney, viz. diabetic nephropathy and polycystic kidney disease.

1587 SPHINGOLIPIDS AS MEDIATORS OF TOXIN-INDUCED CELLULAR DEREGULATION

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Sphingolipids are bioactive molecules which play critical roles in regulating cell behavior. It was hypothesized that disruption of sphingolipid metabolism would alter cell function. Fumonisins (FB) are a family of mycotoxins which are toxic and carcinogenic for animals and perhaps humans. They have as their molecular target, ceramide synthase, the enzyme in the *de novo* sphingolipid biosynthetic pathway which acylates sphingoid bases to form ceramides. FB-inhibition of this enzyme causes accumulation of sphingoid bases and depletion of more complex sphingolipids. Using Swiss 3T3 fibroblasts as a model, it was found that FB1 stimulates DNA synthesis. Furthermore, the addition of β-haloalanines, which reduces FB-induced accumulation of sphingoid bases, prevents FB-induced mitogenesis. In other cell-types, FB disruption of sphingolipid metabolism can be growth inhibitory. For example, in LLC-PK1 cells, both the accumulation of sphingoid bases and the reduction in more complex sphingolipids contributes to reduced cell growth and increased cell death and these effects can be partially reversed by the addition of β-haloalanines. These findings established that disruption of sphingolipid metabolism by toxins can lead to cellular deregulation. Supported by USDA #93-37204-9339.

1588 TOXIN-INDUCED DISRUPTION OF SPHINGOLIPID METABOLISM AND ITS ROLE IN ANIMAL DISEASE

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Fungal metabolites with structural similarity to sphinganine, an intermediate in the biosynthesis of other long-chain bases and complex sphingolipids, are known to be produced by eight genera of fungi. Two enzymes in the sphingolipid biosynthetic pathway have been identified as targets for these metabolites: serine palmitoyl-transferase (SPT) and ceramide synthase. While the activity of SPT is clearly required for cell growth, only the inhibitors of ceramide synthase (fumonisins [FBs]and AAL-toxins) are associated with disease in eucaryotic organisms. The AAL-toxins are extremely phytotoxic but have never been associated with animal disease. The FBs are known to cause pulmonary edema in pigs and leukoencephalomalacia in horses, and have been implicated in other farm animal diseases and in human cancer. FBs

have been reported to be hepatotoxic, nephrotoxic, and hepatocarcinogenic in rats. When pigs, horses, or rats are fed FBs, sphinganine accumulates in liver and kidney and soon thereafter appears in blood and (in rat) urine. Complex sphingolipids in these tissues decrease. The increase in serum sphinganine precedes liver pathology and closely parrallels the increase in serum enzymes indicative of liver injury. In rat, the increase in free sphinganine concentration in urine is correlated with the extent of kidney pathology. However, disrupted sphingolipid metabolism is also observed in tissues which are not considered target organs. Recently, FB1 has been shown to disrupt endothelial cell function. Thus, FB-induced disruption of vascular function could contribute indirectly to the tissue damage associated with exposure to FBs.

1589 ACETYLCHOLINESTERASE (AChE) AND NEUROPATHY TARGET ESTERASE (NTE) INHIBITION IN NEUROBLASTOMA CELLS IDENTIFIED ORGANOPHOSPHORUS COMPOUNDS (OPs) MORE LIKELY TO CAUSE ACUTE RATHER THAN DELAYED NEUROTOXICITY (OPIDN)

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Concentration-related inhibition of NTE and AChE were determined in human (SH-SY5Y) and mouse (NB41A3) neuroblastoma cells exposed to neuropathy-inducing OPs [mipafox, diisopropylphosphorofluoridate (DFP), phenyl saligenin phosphate (PSP), tolyl saligenin phosphate (TSP)], non-neuropathic OPs [paraoxon, malaoxon], and OPs that may cause OPIDN under special circumstances [dichlorvos, chlorpyrifos-oxon]. IC50 ratios for NTE inhibition/AChE inhibition were < 12 for mipafox, DFP, PSP, and TSP; > 2000 for malaoxon and paraoxon; and between 15 and 140 for dichlorvos and chlorpyrifos-oxon. The ratio of NTE /AChE IC50's was lower in human cells than in mouse cells for dichlorvos (16 versus 137) and chlorpyrifos-oxon (23 versus 39), suggesting some species differences. The results indicate that an *in vitro* system of neuroblastoma cells could determine which OPs are more likely to cause an acute cholinergic crisis and which are more likely to cause OPIDN in humans and animals (Supported by USEPA HERL).

1590 EFFECT OF *IN VIVO* AND *IN VITRO* EXPOSURE TO CHLORPYRIFOS OR CHLORPYRIFOS OXON ON RAT LIVER AND BRAIN CARBOXYLESTERASE AND ACETYLCHOLINESTERASE

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It is well-known that the toxicity of anticholinesterase pesticides may vary according to the age or sex of the exposed population; however, the mechanism(s) for this selectivity is not well-defined. It is postulated that non-target esterases, such as carboxylesterases (CaE), may play a role in conferring this selectivity. Organophosphate pesticides (OPs) can bind to CaE which may lower the concentration of OPs at the target site enzyme, acetylcholinesterase (AChE). The role of CaE in modifying the degree of toxicity following exposure to the OP pesticide, chlorpyrifos (CPF) was investigated. Adult rats were exposed to 80 mg/kg, p. o. or vehicle (corn oil), sacrificed at 1, 2,3.5,6.5,24,72,168, or 336 hrs (n = 5/timepoint/dose). AChE and CaE activity were measured in the brain and perfused liver. In the brain, AChE was more inhibited (50-80%) than CaE (30-40%) from 1 to 24 hrs, after which the AChE recovered at a faster rate than the CaE. In the liver, both AChE and CaE were >80% inhibited from 1 to 24 hrs after which they recovered; again, AChE recovered at a faster rate than CaE. The *in vitro* sensitivity of brain CaE and AChE mimicked the pattern of inhibition seen after *in vivo* dosing with CPF: brain AChE ($IC_{50} \approx 3$ nM) was approximately 30 times more sensitive to CPFoxon than was brain CaE ($IC_{50} \approx 118$ nM). Interestingly, that pattern was reversed in hepatic tissue: liver AChE ($IC_{50} \approx 2{,}500$ nM) is about 2500 times *less* sensitive to CPFoxon than is liver CaE ($IC_{50} \approx 0.89$ nM). The data indicate that (1) the *in vitro* sensitive of CaE or AChE to CPFoxon is highly tissue specific and (2) brain CaE does not "protect" brain AChE from inhibition by CPF or its oxon.

1591 ORGANOPHOSPHATE-INDUCED MATERNAL AND PUP CHOLINESTERASE INHIBITION IN TWO-GENERATION REPRODUCTION STUDIES WITH RATS

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Six organophosphate (OP) esters (azinphos methyl, coumaphos, fenamiphos, oxydemeton methyl, tribufos, and trichlorfon), which inhibit cholinesterase and are used as insecticides or as a defoliant (tribufos), have been evaluated in two-generation reproduction studies with rats. In these studies, the cholinesterase activity in plasma, erythrocytes, and brain was evaluated in dams, 4-day old pups, and 21-day old pups. For each compound, the highest dose tested was maternally toxic and caused inhibition of cholinesterase activity which ranged from 54–93% in plasma, 33–91% in erythrocytes, and 29–81% in brain. This was in contrast to 4-day old pups which had no cholinesterase inhibition or slight cholinesterase inhibition relative to the dam at the highest dose tested. In 21-day old pups, which have begun to eat solid food, some significant cholinesterase inhibition was observed, but was always lower than the maternal cholinesterase inhibition. At lower doses, whether or not significant maternal cholinesterase inhibition was observed, no cholinesterase inhibition was observed in 4-day old pups. These results show that even with a high maternal body burden of the OP esters tested (as reflected by high cholinesterase inhibition) the exposure of nursing pups to the OP was either negligible or significantly lower than the dam, and at lower body burdens nursing pups experienced no cholinesterase inhibition. In conclusion, cholinesterase inhibition in dams is the most sensitive endpoint for toxicity in both dams and pups.

1592 SUBCHRONIC NEUROTOXICITY SCREENING STUDY RESULTS WITH SIX ORGANOPHOSPHATE INSECTICIDES: THE UTILITY OF VARIOUS INDICES TO ASSESS NEUROTOXIC POTENTIAL

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The organophosphate (OP) insecticides sulprofos, disulfoton, azinphos-methyl, methamidophos, trichlorfon, and tebupirimphos were tested for neurotoxicity, in accordance with U.S. EPA (FIFRA) guideline 82–7, to support product registrations. Each OP was administered through the diet for 13 weeks to young-adult Fischer 344 rats at four dose levels, including a vehicle control. For each OP, 12 rats/sex/dietary level were tested using a functional observational battery (FOB), an automated measure of activity (figure-8 maze) and clinical observations, with six/sex/dose level perfused at term for microscopic examination of neural tissues. Erythrocyte, plasma and brain cholinesterase activities (ChE) were measured using an additional six rats/sex/dose level.

With each OP, ChE was the most sensitive index of exposure and additional cumulative toxicity was generally not evident after four weeks of exposure. Furthermore, the FOB and motor activity findings are consistent with clinical observations and occurred at comparable dose levels. These results support (1) the value of detailed clinical observations and other tests that are routinely used in toxicology studies to screen for neurotoxicity, (2) a standard of > 20% inhibition of ChE for biological significance and (3) the use of ChE inhibition to establish the No-Observed-Effect Level (NOEL) for OPs.

1593 COMPARATIVE CHANGES IN HIGH AFFINITY CHOLINE UPTAKE AND [^{3}H] HEMICHOLINIUM-3 BINDING IN RAT BRAIN FOLLOWING PARATHION AND CHLORPYRIFOS EXPOSURE

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Organophosphorus pesticides exert toxicity through inhibition of acetylcholinesterase (AChE). Previous studies in our laboratory have demonstrated, however, that doses of parathion (PS) and chlorpyrifos (CPF) which cause similar changes in AChE activity can produce marked differences in toxicity. While compensatory changes in postsynaptic receptors can modulate the expression of acetylcholinesterase inhibition, we propose that selective changes in presynaptic processes can also contribute to the differential toxicities of these AChE inhibitors. We examined the relative effects of either PS (6.6 mg/kg, sc) or CPF (280 mg/kg, sc) on high affinity choline uptake (HACU), the rate-limiting step in acetylcholine synthesis, 1–7 days after exposure. Female rats treated with PS or CPF exhibited similar maximal degrees of brain AChE inhibition (82–95%) but PS produced more extensive signs of acute toxicity (i.e. SLUD signs and involuntary movements were

greater following PS). CPF decreased synaptosomal HACU (13–16%) at 1, 2 and 7 days after exposure. PS also reduced HACU (22%) but only at 2 days after treatment. Using [^{3}H] hemicholinium-3 (HC-3) to label the choline transporter, dose-related reductions in HC-3 binding and AChE activity were noted 1 day after CPF exposure. Kinetic analyses indicated a significant change in Bmax (from 187 to 103 fmol/mg protein) but no change in binding affinity with HC-3. As acute toxicity was maximal within the first day following either pesticide, early changes in choline transport following CPF exposure may alter acetylcholine synthesis and thereby contribute to the relative differences in toxicity with these two agents.

1594 BRAIN REGIONAL VARIABILITY IN CHOLINERGIC AND NONCHOLINERGIC BIOMARKERS: IN VIVO ACUTE EFFECTS OF ACHE INHIBITORS

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The major objective of this study was to determine the distribution of AChE, CK, and LDH, and their isoenzymes in discrete rat brain regions. Activities of AChE and LDH were found highest in striatum and of CK in cerebellum. CK isoenzymes in stem, striatum, and hippocampus were very similar (CK-1 86.2–88.5%; CK-2 2.8–5.1%; and CK-3 6.7–9.4%). Cortex, however, had lowest CK-1, and highest CK-3. CK-3 in all brain areas had only the CK-MM3 subform. Each brain area presented a characteristic profile of LDH isoenzymes, with LDH-4 the highest (23–40%), except in cerebellum which had maximum LDH-1 (29%). The other objective was to characterize the changes induced by carbofuran (1.5 mg/Kg, sc) or methyl parathion (MPTH, 5 mg/Kg, ip). Maximal AChE inhibition occurred in cortex with carbofuran and in cerebellum with MPTH, and least in striatum with either insecticide. Both insecticides caused characteristic alterations in CK, LDH, and their isoenzymes; and their leakage into circulation by increased membrane permeability due to depletion of ATP (39–54%) and PCr (21–27%).

1595 DIRECT EFFECTS OF ORGANOPHOSPHATES AND PHYSOSTIGMINE ON NEURONAL ACETYLCHOLINE RECEPTORS OF DIFFERENT SPECIES

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Organophosphate (OP) neurotoxicity exhibits pronounced species-specificity. Although OPs are generally known to affect esterases, acetylcholine (ACh) receptors have also been proposed as possible target sites.
The effects of parathion, paraoxon, PSP and physostigmine were analyzed on neuronal nicotinic receptors in mouse N1E-115 cells, human SH-SY5Y cells and locust neurons. In all cells ACh induced a similar nicotinic respons, that was inhibited by OPs at micromolar concentrations. Block differed depending on the species origin of the cells and the compound tested. The potency of block followed the same order in each cell type: parathion > physostigmine > PSP > paraoxon. Surprisingly, the nicotinic receptor is more sensitive to parathion compared to the active metabolite paraoxon. Depending on the cell type, a 15–50 fold potency difference was observed. The effects of OPs were also examined on human muscarinic M3 receptors, transfected in chinese hamster ovary (CHO) cells. ACh induced a muscarinic outward current and also increased intracellular calcium. In 20–35% of the responding cells parathion and paraoxon activated an ion current or paraoxon increased intracellular calcium. However, these preliminary results indicate that the OPs affect the muscarinic receptor in a different manner then ACh.
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1596 TISSUE AND AGE-RELATED SENSITIVITY OF RAT PLASMA AND LIVER ACETYLCHOLINESTERASE TO CHLORPYRIFOS

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Young animals exhibit an increased susceptibility to the lethal effects of cholinesterase (ChE)-inhibiting insecticides including chlorpyrifos (CPF), although the mechanism(s) for such sensitivity is unknown. Although our previous studies indicate that young (PND4) rat brain acetylcholinesterase (AChE) is not intrinsically more sensitive to CPFoxon, the sensitivity of plasma and liver AChE is unknown. We first characterized the ChEs of plasma and perfused liver of PND4 ($n \geq 3$) and adult ($\geq$90 day, $n \geq 3$) male Long Evans rats. In both tissues, there was a decrease in the proportion of AChE activity (relative to butyrylcholinesterase activity) as the animal developed. Secondly, we examined the sensitivity of the AChE in plasma or perfused liver to CPFoxon: IC_{50}s (30 min at 26°C) were defined concomitantly for PND4 and adult plasma or liver AChE. Both plasma and liver AChE IC_{50}s showed age-related differences: PND4 $\approx$ 6 nM and adult $\approx$ 44 nM for plasma, and PND4 $\approx$ 590 nM and adult $\approx$ 3000 nM for perfused liver. Because IC_{50} values may be overestimated when Ca^{2+}-dependent hydrolases (Ca^{2+}-DH) are not taken into consideration, the AChE IC_{50}s were also determined in the presence of 1 mM EGTA to inhibit these hydrolyases. These results showed that Ca^{2+}-DH activity increased the apparent IC_{50} values for the plasma at both ages; the adult plasma showed more of a shift than the PND4 plasma. In contrast, Ca^{2+}-DH activity did not appear to be a factor in modifying the IC_{50} values for the perfused liver. In summary, there is a significant tissue and age-related difference in the sensitivity of plasma and liver AChE to CPFoxon.

1597 PROTECTION AND REVERSAL OF CARBOFURAN-INDUCED BIOCHEMICAL CHANGES IN DIAPHRAGM MUSCLE BY MEMANTINE AND ATROPINE

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We demonstrated for the first time that a combined antidotal treatment with memantine (MEM, 18 mg/kg, sc) and atropine (ATS, 16 mg/kg, sc) provided complete protection against carbofuran toxicity (1.5 mg/kg, sc) in rats by protection and/or reversal of inhibited AChE and CarbE. Carbofuran not only inhibits esterases but also perturbs the activities of mitochondrial/cytoplasmic (CK and LDH) biomarker enzymes and their isoenzymes patterns in target organs. Diaphragm muscle showed the most profound changes. Characteristic changes in the activity of biomarker enzymes/isoenzymes were reflected in serum as a result of their leakage from diaphragm due to depletion of ATP (27%) and PCr (33%). MEM and ATS provided protection and reversal of the induced changes in biomarkers by preventing depletion of high-energy phosphates and thereby maintaining normal cell membrane characteristics, including permeability and integrity. These results, along with those reported previously, indicate that MEM antagonizes carbofuran toxicity by multiple mechanisms.

1598 TRIPHENYLPHOSPHINE (TPP): A TYPE III ORGANOPHOSPHORUS COMPOUND-INDUCED DELAYED NEUROTOXIC AGENT (OPIDN)

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While most organophosphorus compounds exert their acute toxicity by inhibiting acetylcholinesterase (AChE) in the nervous system, some of these compounds produce a neurodegenerative disorder known as organophosphorus compound-induced delayed neurotoxicity (OPIDN). Pentavalent organophosphorus compounds, such as tri-*o*-cresyl phosphate and diisopropyl phosphorofluoridate produce Type I OPIDN characterized by ataxia and paralysis after a 1–2 week delay period accompanied by Wallerian-like degeneration of the axon followed by secondary myelin degeneration. Trivalent organophosphorus compounds such as triphenyl phosphite produce Type II OPIDN which differs from Type I by rodents sensitivity and neuropathological lesions in neuronal cell bodies. A single oral dose of 5 g/kg of triphenylphosphine (TPP) neither produced any clinical signs of toxicity in hens nor did it inhibit brain AChE or neurotoxic esterase (NTE), but caused inhibition of plasma butyrylcholinesterase activity. Daily oral administration of 500, 2,000, or 5,000 mg/kg TPP for 4 consecutive days resulted in ataxia and paralysis that progressed to death in high doses. Spinal cord and sciatic nerves exhibited neuropathologic lesions identical in morphology and distribution to OPIDN. There was severe damage to the ventral tracts, with more moderate damage to ventral lateral and lateral tracts. In the most severe cases, enlarged axon can be seen in the dorsal horns. Peripheral nerve damage ranges from severe to mild. The moderate to mild cases are more common in the lower dose group. It is concluded that inhibition of esterases, i.e., AChE or NTE is not involved in the pathogeneses of triphenyl phosphine-induced delayed neurotoxicity that may constitute a Type III OPIDN that is distinct from the other two types. (Supported in part by NIEHS grant ESO 5154.)

1599 POTENTIATION OF NO_2-INDUCED OXIDATION BY LUNG LINING LAYER ANTIOXIDANTS

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Absorption of inhaled NO_2 is mediated by reaction with pulmonary epithelial lining fluid (ELF) constituents. Kinetic studies of NO_2 gas phase disappearance suggest that the preferential ELF substrates which drive NO_2 absorption are glutathione (GSH) and ascorbate (AH_2). We have utilized a 3 compartment model of the lung surface to investigate whether these absorption substrates potentiate exposure-induced membrane oxidation. Red cell membranes (RCM), covalently bound to glass substrata, were covered with model ELF or rat bronchoalveolar lavage fluid (BALF) and exposed to NO_2 (10 ppm; 20–30 min) with cyclic tilting. RCM oxidation was assessed via ELF thiobarbituric acid reactants (TBARS) and RCM protein sulfhydryls (PSH). **Results:** 1) Exposure-induced oxidation of RCM required the presence of ELF substrates (GSH, AH_2, or BALF). The time-course and extent of oxidation was substrate concentration-dependent. 2) Depletion of BALF GSH (*N*-ethylmaleimide) and AH_2 (ascorbate oxidase) substantially reduced RCM oxidation. 3) Egg phosphatidylcholine (Egg PC) liposomes induced modest membrane oxidation. Addition of GSH or AH_2 to Egg PC produced profound RCM oxidation. 4) RCM oxidation was superoxide dismutase inhibitable for GSH but not AH_2. **Conclusions:** ELF GSH and AH_2 likely display prooxidant properties and initiate secondary oxidative events during NO_2 exposure. GSH reactions potentially lead to O_2 production. ELF lipids may play an intermediate role in induction of secondary membrane oxidation. (CIAR 90–23, NIH ES04952 & T32ES07254, and Meadows Foundation)

1600 1, 2-DIHALOETHANE METABOLISM AND OXIDATIVE STRESS: THE ROLES OF GLUTATHIONE AND VITAMIN E

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Severe depletion of cellular glutathione can be accompanied by loss of vitamin E levels especially when the glutathione depletion includes the depletion of mitochondrial glutathione. The question is whether exogenous oxidative stress creates intracellular conditions that cause the normally protective glutathione and vitamin E to become contributors to endogenous oxidative stress leading to loss of these constituents and possible participation in oxidative events including lipid peroxidation and loss of protein thiols. Rat hepatocytes in suspension culture, have been exposed to chemicals not known to generate free radicals directly; 8.8 mM 1,2-dichloroethane (DCE), or 0.1 to 0.5 mM 1,2-dibromoethane (DBE). Vitamin E and cytosolic and mitochondrial GSH levels were measured with time up to 4–6 h. Depletion of GSH was accompanied by loss of vitamin E, increased lipid peroxidation and loss of protein thiols. These data support the suggestion that depletion of mitochondrial GSH was a principal indicator of the loss of control of endogenous oxidative stress. We conclude that, upon severe depletion, the protective systems of GSH and vitamin E may contribute to the overall oxidative stress due to lack of reducing equivalents needed to prevent their loss. In all of our studies with rat hepatocytes, oxidative stress becomes a serious contributor to cell injury and death when mitochondrial GSH is depleted by about 50% suggesting the loss of supply of reducing equivalents to the protective systems. Also, that vitamin E may contribute to the free radicals participating in cellular damage as indicated by decreased cellular concentration presumably by radical interaction with cellular constituents.
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1601 REACTIVE PHENOXYL RADICALS CAUSE CYTOTOXICITY OF PHENOLIC COMPOUNDS

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Effectiveness of phenolic antioxidants in protecting against oxidative stress depends on their reactivity towards reactive oxygen species and the reactivity of the antioxidant phenoxyl radicals towards critical biomolecules. Efficient scavenging of reactive oxygen species may result in toxic rather than protective effects if reactive phenoxyl radicals oxidatively modify DNA, proteins or lipids. We report that phenoxyl radicals formed from several phenolic compounds (e.g., phenol, a phenolic antitumor drug, Etoposide) which are very efficient in protecting DNA and lipids selectively oxidize protein sulfhydryls and GSH. These prooxidant effects of phenolics were observed in model systems (e.g., tyrosinase/phenolic compound + GSH or SH-containing proteins), as well as in cells (e.g., in myelotic human leukemia HL60 cells) and animal tissues (e.g., in heart and lung of rats treated with a single ip injection of etoposide, 10 mg/kg). In addition, reactive phenoxyl radicals may attack polyunsaturated lipids as evidenced by the phenoxyl radical-induced oxidation of *cis*-parinaric acid. Thus, reactivity of phenoxyl radicals should be considered as a critical factor in the development of new antioxidant protectants.

1602 A NOVEL ANTIOXIDANT FUNCTION OF NITRIC OXIDE AGAINST OXIDATIVE DAMAGE INDUCED BY HEMOGLOBIN

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Interaction of hemoglobin (Hb) with H_2O_2 or organic hydroperoxides may cause oxidative stress. One of the pathophysiological condition associated with increased free Hb is hemorrhage where infiltrate is lysed with the subsequent release of Hb. Using EPR technique, we found two reactions acting independently or consequently to yield oxidants responsible for oxidative stress induced by Hb and tert-butyl hydroperoxide (tBuOOH): one of these reactions (the faster) produces tBOO• from metHb/tBuOOH by one electron oxidation of tBuOOH, the second reaction (the slower) results in the production of oxoferrylHb species by two electron oxidation of heme iron. The production of tBOO• and protein radi-cals were also found upon interaction of tBuOOH with endogenous Hb in human leukemia K562 and K/VP.5 cells. The observed cytotoxic effects of tBuOOH was proportional to endogenous Hb content which was manipulated by growing the cells in the presence of exogenous hemin. Similarly, the extent of oxidative stress, assessed by measuring fluorescent probe, cisparinarioyl-residues, incorporated in membrane phospholipids of K562 and K/VP.5 cells, was also dependent on the endogenous Hb levels. In search for protection from Hb/tBuOOH-induced oxidative stress, we found that nitric oxide (NO) was efficient in reducing oxoferrylHb and associated peroxidation reactions. These results represent a novel antioxidant mechanism by which NO may play an important role in protecting cells and tissues from Hb-induced oxidative damage.

1603 ROLE OF ANTIOXIDANTS IN FREE RADICAL MEDIATED TOXICITY OF COPPER: AN ESR SPIN TRAPPING INVESTIGATION

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Many laboratory and clinical studies suggest that free radical generation and resultant cell damage contribute to liver injury following copper poisoning or genetic copper diseases. Accordingly, antioxidants represent a viable therapeutic approach for management of oxidative damage. The ESR spin trapping technique has been used to investigate free radical generation in copper-challenged rats deficient in vitamin E and/or selenium and also in rats treated simultaneously with copper and ascorbic acid. Lipid-derived radical adducts of α-phenyl-*N-tert*-butylnitrone (PBN) was detected in the bile of rats deficient in both vitamin E and selenium whereas the methyl radical adduct of PBN was detected after acute copper and ascorbic acid intake. Bile samples were collected into chelating agents in order to prevent *ex vivo* of radical adduct generation in bile during its collection. The findings that copper causes little detectable radical adduct formation in rats fed selenium-and vitamin E-supplemented diet support the proposal that dietary selenium and vitamin E can protect against lipid peroxidation and copper toxicity. In addition, the results of this study support a free radical mechanism that attribute an important *in vivo* role to ascorbic acid in the induction of biological damage already demonstrated in numerous *in vitro* experiments.

1604 UVB-INDUCED VITAMIN E RADICAL DOES NOT PROPAGATE LIPID PEROXIDATION IN LOW DENSITY LIPOPROTEINS AND IN SKIN

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Vitamin E plays a critical role in wellness, health maintenance and in the prevention of chronic and degenerative diseases. Actually it is a very dynamic molecule involved in a series of antioxidant cycles. This occurs through the interactions between water- and lipid- soluble substances, by non-enzymatic and enzymatic mechanisms, which regenerate vitamin E from its radical form to the initial antioxidant form. In contrast to the well recognized function of vitamin E as membrane antioxidant, recent studies suggested that vitamin E may act as a prooxidant in low density lipoproteins (LDL) and in the skin

via the mechanism of vitamin E-radical- mediated propagation of lipid peroxidation. To experimentally assess this hypothesis we generated vitamin E radical in LDL by UVB-irradiation and compared the UVB-induced loss of vitamin E with accumulation of hydroperoxides. To eliminate the autooxidation reactions we added deferoxamine. No accumulation of hydroperoxides was observed during the initial time of UVB-irradiation of LDL, whereas the level of vitamin E decreased more than 5-fold. In the absence of deferoxamine, vitamin E loss proceeded with a higher rate and the accumulation of hydroperoxides occurred from the beginning and its rate increased 4.3 fold for cholesteryl ester hydroperoxides and 2.9 fold for phospholipid hydroperoxides respectively, after the depletion of vitamin E. Similar results were obtained in skin homogenates during the UVB-irradiation.
We conclude that in the absence of transition metals, vitamin E and its phenoxyl radicals are not likely to participate in propagation of lipid peroxidation.

1605 REDOX RESPONSES PRECEDING ENZYME INDUCTION AND APOPTOSIS IN HUMAN COLON HT29 CELLS

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Redox changes have been suggested to be involved in both the induction of detoxication enzymes and apoptosis. We previously found that benzyl isothiocyanate (BIT) induced the anticarcinogenic enzymes glutathione S-transferase and quinone reductase and also induced apoptosis in HT29 cells. This indicates that colon cells may have two responses to dietary components which protect against carcinogenesis. Recent studies have shown that apoptosis is inhibited by extracellular thiols, suggesting that apoptosis may be modulated therapeutically. However, such changes could interfere with induction of detoxication enzymes if redox changes are involved in their induction. We used BIT to examine how intracellular GSH responds to an inducing agent prior to induction of enzymes and apoptosis. Cells cultured for 3 d were exposed to 25 μM BIT and harvested beginning 15 min after exposure. During the first hour, GSH concentration fell to about 25% of pretreatment values. GSH returned to pretreatment values after about 3 h and increased to 500% of initial values by 6 h. Thus, the results indicate that the response to enzyme inducers involves a biphasic change in GSH levels. If redox changes are involved in signaling, it is not clear whether the initial fall or the subsequent overshoot functions in signaling. Therapeutic interventions designed to inhibit apoptosis by increasing thiol levels may have the effect of also inhibiting other protective mechanisms against carcinogens or oxidative stress.

1606 PROOXIDANT ACTION OF WATER SOLUBLE ANTIOXIDANTS AND THEIR ROLE IN BLAST OVERPRESSURE-INDUCED OXIDATIVE STRESS

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We have shown previously that exposure of rats to blast overpressure (BOP) results in oxidative stress characterized by: (i) progressive depletion of total antioxidant reserve (glutathione (GSH), ascorbate, and vitamin E) (ii) oxidation of protein thiols, (iii) accumulation of lipid peroxidation products, and (iv) formation of methemoglobin (metHb) from oxyhemoglobin (oxyHb). Antioxidants are traditionally associated with protection against oxidative insult. We now report that antioxidant may contribute to enhancement of oxidative stress via redox-cycling of oxyHb/metHb/ferrylHb released by BOP from ruptured red blood cells. Our *in vitro* experiments showed that a two-electron reduction of oxygen in oxyHb by ascorbate and/or GSH yields metHb, H_2O_2 and a one electron oxidation products of the antioxidant, semidehydroascorbyl or thiyl radicals, respectively. Interaction of metHb with H_2O_2 was most likely responsible for the degradation of Hb heme and release of free iron (potentially available for other redox-cycling reactions). Thus, prooxidant action of water-soluble antioxidants via redox-cycling of hemoproteins and non-heme iron may contribute to activation of oxidative stress. The data obtained imply that the use of antioxidants as protectants against Hb-induced oxidative stress may not be unconditional but rather should rely on exact knowledge of the redox-mechanisms involved. Development of mechanism-based protective strategies against BOP-induced damage may require ligands to prevent redox reactions of Hb (e.g., nitric oxide) in combination with the antioxidants.

1607 THE CHEMICAL REACTIVITY OF FERRYLMYOGLOBIN TOWARDS ANTIOXIDANTS. A POTENTIAL ROLE FOR FERRYLMYOGLOBIN IN MUSCLE OXIDATIVE INJURY

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Current interest in the mechanisms by which reactive species cause cellular injury in muscle tissue has focused on the potential damaging role of the reaction between myoglobin -the major heme-containing protein of cardiac and skeletal muscle-and H_2O_2, the product of which is known as ferrylmyoglobin. The latter species contains two oxidation equivalents: an oxoferryl complex ($Fe^{IV}=O$) and a transient protein radical. The oxoferryl complex is a long-lived, stable oxidant with a reduction potential of about 1 V. The reactivity of the ferryl complex towards antioxidants, such as ascorbate, vitamin E, and thiols has been examined. These reactions are complex involving usually one-electron transfer steps with primary formation of the free radical form of the antioxidant. Accordingly, ascorbyl-, chromanoxyl-, and thiyl radicals are formed during the interaction of ferrylmyoglobin with the aforementioned antioxidants. In addition to thiyl radicals, the reaction of thiols with ferrylmyoglobin encompasses transfer of reducing equivalents from the thiol to the iron-ligand complex (sulfur nucleophilic attack on the pyrrole) resulting in sulfmyoglobin formation, a nonfunctional form of the hemoprotein. Alternatively, inactivation of the hemoprotein occurs because the above redox transitions are not unidirectional: they participate in processes of cycling properties centered on the $Fe^{III} \Leftrightarrow Fe^{IV}=O$ transition and involving the reduction of H_2O_2 to H_2O and the oxidation of the reducing agent. Repeated cycles bring about a diminished reactivity of the hemoprotein towards H_2O_2 associated or not with an oxidative modification of the porphyrin.
The pathophysiological role of this species -with implications for cardiac and skeletal muscle oxidative injury- requires consideration of the oxidative reactions that ferrylmyoglobin can trigger as well as the mechanisms participating in its recovery.

1608 EVALUATION OF THE PRINCIPAL HUMAN HEPATIC P450 ISOENZYMES RESPONSIBLE FOR THE BIOTRANSFORMATION OF BESIPIRDINE (HP 749) IN VITRO

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This study was performed to indicate the principal human hepatic cytochrome P450 isozyme(s) responsible for the biotransformation of besipirdine hydrochloride (HP 749), a compound currently under development for the treatment of Alzheimer's disease. Utilizing a bank of ten characterized human liver microsomal preparations, each with different proportions of 10 different P450 isozymes, there was a three fold variation in the biotransformation of HP 749. The disappearance of parent compound ranged from 31% to 97% ,depending on the donor microsomal preparation. Correlation analysis revealed a significant correlation of HP 749 conversion with CYP2A6 activity ($r = 0.830$, $P < 0.01$). HP 749 biotransformation was partially inhibited by monoclonal anti-2A6 antibody and HP 749 was a substrate for recombinant lymphoblastoma microsomes expressing only human CYP2A6. Whereas CYP3A activity correlated to a lesser and statistically non-significant extent with HP 749 biotransformation ($r = 0.479$) in ten donor tissues, anti-CYP3A4 antibody inhibited disappearance of parent by 24.4%, and HP 749 was a substrate in lymphoblastoma microsomes expressing only CYP3A4. These studies indicate that cytochrome 2A6 is likely to be a principal isozyme responsible for the hepatic biotransformation of HP 749 in man *in vivo*, and that CYP3A4 may also play a role due to its capacity to utilize HP 749 as a substrate and its high relative expression in human liver.

1609 LIPOIC ACID IMPAIRS GLYCINE CONJUGATION OF BENZOIC ACID AND RENAL EXCRETION OF BENZOYLGLYCINE

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Glycine conjugation of benzoic acid is catalyzed by the mitochondrial

enzymes benzoyl-CoA synthetase and benzoyl-CoA:glycine *N*-acyltransferase and requires ATP, coenzyme A and glycine as cosubstrates. Because lipoic acid also forms a coenzyme A ester, it may interfere with glycine conjugation. To test this hypothesis, lipoic acid (0.5–1.5 mmol/kg ip) was given to anesthetized, glycine-loaded rats 1 hr before administration of benzoic acid (1 mmol/kg iv). Lipoic acid inhibited glycine conjugation of benzoic acid in a dose-dependent manner as indicated by (1) reduced clearance of benzoic acid from blood, (2) delayed appearance of benzoylglycine in blood, and (3) decreased excretion of benzoylglycine in urine. Lipoic acid also decreased urinary excretion of injected benzoylglycine indicating that reduced excretion of this metabolite following benzoic acid injection is due to diminished formation and impaired renal transport of benzoylglycine. Urine formation was decreased by lipoic acid in a dose-dependent fashion and acute renal failure was evident in rats receiving the highest dose. Lipoic acid depleted hepatic coenzyme A, carnitine and glutathione, but not ATP and glycine and inhibited benzoyl-CoA synthetase (IC_{50} 1.5 mM) as well as benzoyl-CoA:glycine *N*-acyltransferase (IC_{50} 0.3 mM). Thus, depletion of coenzyme A and inhibition of the pertinent enzymes appear responsible for impairement of glycine conjugation by lipoic acid. Lipoic acid may also inhibit the citric acid cycle in the renal tubular cells resulting in impaired renal transport of benzoylglycine and acute renal failure.

1610 CO-OXIDATIVE METABOLISM OF 4-AMINOBIPHENYL (4-ABP) BY LIPOXYGENASE FROM SOYBEAN (SLO) AND HUMAN TERM PLACENTA (HTPLO)

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Many reports have documented the transfer of 4-ABP across human placenta. The presence of hemoglobin and DNA adducts of 4-ABP in placenta and cord blood is also observed. Since the mechanism of placental 4-ABP bioactivation is unknown, linoleic acid (LA)-dependent co-oxidation catalyzed by HTPLO was studied. SLO, a model LO, was used for comparison. Spectral analyses of reaction media containing 4-ABP, LA and SLO/HTPLO suggested the disappearance of substrate (ΔA at 270 nm) and a gradual appearance of a new peak at 315 nm, indicating a metabolite formation. Under optimal assay conditions, SLO exhibited a specific activity of 350 nmoles of 4-ABP depleted/min/nmole of enzyme. To observe the maximal rate of co-oxidation by HTPLO (45 nmoles of 4-ABP depleted/min/mg protein), incubation of 50 μM 4-ABP, 2 mM LA and 80 μg/ml protein at pH 7.4 was essential. 4-ABP was also oxidized by SLO in the presence of H_2O_2. The previous report on the production of azobis-ABP during H_2O_2-dependent horseradish peroxidase catalyzed oxidation of 4-ABP was confirmed by reverse-phase HPLC. The similarity in the retention time suggests a possibility that azobis-ABP is also produced by SLO/HTPLO. Both SLO and HTPLO mediated reactions were significantly inhibited by NDGA, BHT, BHA, gossypol and ETI. These results suggest that the co-oxidation catalyzed by HTPLO may be the underlying biochemical mechanism responsible for the transplacental toxicity of 4-ABP.

1611 *N*-ACETYL-S-(1,2,2-TRICHLOROVINYL)-L-CYSTEINE AND 2,2,2-TRICHLOROETHANOL: TWO NOVEL METABOLITES OF TETRACHLOROETHENE IN HUMANS AFTER OCCUPATIONAL EXPOSURE

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The excretion of tetrachloroethene metabolites in urine was studied in occupationally exposed workers (dry cleaners) in order to identify and quantify metabolites. Tetrachloroethene concentrations in the air of the dry cleaning shop were 50 ± 4 ppm; 2 individuals were exposed for 8 h daily and 2 for 4 h daily. In urine samples collected from the individuals at the beginning and at the end of the work week, *N*-acetyl-*S*-(1,2,2-trichlorovinyl)-L-cysteine and 2,2,2-tri-chloroethanol were identified by GC/MS as tetrachloroethene-metabolites in humans. The concentrations of *N*-acetyl-*S*-(1,2,2-tri-chlorovinyl)-L-cysteine in the urine of the individuals were not significantly different at the start and at the end of the work week; however, concentrations of both *N*-acetyl-*S*-(1,2,2-trichlorovinyl)-L-cysteine and 2,2,2-trichloro compounds (trichloroacetic acid and 2,2,2-trichloroethanol) as markers for GSH- and cytochrome P450-mediated metabolism, resp., were proportional to the length of daily tetrachloroethene exposure. A remarkable difference in the excretion pattern of 2,2,2-trichloro compounds, the major tetrachloroethene metabolites was observed. Trichloroacetic acid and 2,2,2-tri-chloroethanol were present in the urine of two of the exposed individuals. Only 2,2,2-trichloroethanol was identified as a major urinary tetrachloroethene metabolite in two other individuals, not excreting detectable amounts of trichloroacetic acid. The results indicate that humans also have the ability to biosynthesize nephrotoxic glutathione *S*-conjugates from tetrachloroethene; however, when compared to rats, the human capacity for the biosynthesis of *N*-acetyl-*S*-(1,2,2-trichlorovinyl)-L-cysteine seems to be lower.

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1612 PURIFICATION AND CHARACTERIZATION OF AN AMINOACYLASE CATALYZING THE HYDROLYSIS OF N-ACETYL-S-(1,2-DICHLOROVINYL)-L-CYSTEINE

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Aminoacylases catalyze the hydrolysis of *N*-acyl-L-amino acids to give fatty acids and amino acids as products. Aminoacylases participate in conjugation-dependent nephrotoxicity by catalyzing the conversion of haloalkene-derived mercapturates to cysteine *S*-conjugates that may undergo β-lyase-catalyzed bioactivation. Aminoacylases also catalyze the deacetylation of *N*-acetyl-L-cysteine (NAC), which is used in clinical practice. Renal aminoacylase I has been well characterized and shows high activity with *N*-acetyl-L-methionine (NAM) and NAC, but little activity with haloalkene-derived mercapturates, including *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (NADCVC). Rat renal homogenates catalyze the deacetylation of a range of mercapturates, indicating the presence of another enzyme. The objective of the research was to test the hypothesis that multiple aminoacylases catalyze the hydrolysis of mercapturates and to purify the aminoacylase catalyzing the hydrolysis of haloalkene-derived mercapturates. An aminoacylase with high activity with NADCVC and little activity with NAC and NAM was purified about 250-fold from Sprague-Dawley rat kidneys. The purification procedure involved $(NH_4)_2SO_4$ fractionation of the renal cytosolic fractions, DEAE-Cellulose column chromatography, Sephacryl S-200 HR column chromatography, hydroxyapatite column chromatography, and FPLC on a Mono-Q column and provided complete separation of aminoacylase I from the purified protein. SDS-PAGE analysis showed that the purified aminoacylase was homogeneous and has a molecular mass of about 20 kDa compared with aminoacylase I, which is a homodimer with a subunit molecular mass of 43 kDa. The purified aminoacylase may be identical with aminoacylase III, which catalyzes the hydrolysis of a range of mercapturates. (Supported by NIEHS grant ES03127)

1613 RENAL AND HEPATIC METABOLISM AND CYTOTOXICITY OF PERCHLOROETHYLENE (PER) BY GSH CONJUGATION

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Metabolism of Per to the GSH *S*-conjugate *S*-(1,1,2-trichlorovinyl)-glutathione (TCVG) was assessed in isolated hepatocytes, isolated renal cortical cells, and in liver and kidney cytosol and microsomes from male and female F344 rats. TCVG was detected by ion-exchange HPLC on an amine column after derivatization with 1-fluoro-2,4-dinitrobenzene. TCVG formation occurred in both liver and renal cortical cells. Because the kidney contains significant amounts of γ-glutamyltransferase and dipeptidase activities, in contrast to the liver, and also has cysteine conjugate β-lyase activity, this demonstrates the presence of the complete bioactivation pathway for Per in the kidney. Rates of TCVG formation in cells and subcellular fractions from male rats were markedly higher than those in the corresponding material from female rats. However, high amounts of GSH-derived degradation products were recovered in incubations of female rat kidney cells or renal microsomes with Per and GSH, possibly because female rat kidneys contain nearly twice the activity of γ-glutamyltransferase as that in male rat kidneys. Rates of TCVG formation from Per in isolated renal cortical cells were threefold faster than those of GSH conjugate formation from trichloroethylene. Per produced marked acute cytotoxicity in isolated hepatocytes from male F344 rats but was not cytotoxic in either renal cells from male F344 rats or in hepatocytes or renal cells from female F344 rats. These results may aid in understanding of sex- and tissue-dependent differences in susceptibility to Per induced toxicity and carcinogenicity.

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1614 FORMATION OF COTININE DURING HUMAN PLACENTAL TRANSFER OF NICOTINE, ITS RETENTION IN FETAL CIRCULATION AND TOXICOLOGICAL IMPLICATIONS

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Nicotine is oxidized to cotinine which has a long plasma half-life (19-24 hr) in humans. Cotinine activates phospholipase A_2 (PLA_2)-like enzymes resulting in formation of prostaglandins (PGE_2 and $PGF_{2\alpha}$) which induce abortions. Therefore, we have studied the placental transfer of nicotine and its convertion to cotinine using isolated perfused human cotyledon. The cotyledon was perfused with aerated (21% O_2, 5% CO_2) Kreb-Ringer bicarbonate buffer (pH 7.4, 37° C) containing 2% albumin on both maternal (230 ml, 15 ml/min, 0.6'' Hg) and fetal (93 ml 1.75 ml/min, 1.75'' Hg) sides in a closed recirculating system. Nicotine was added to maternal (2 mg) or fetal (0.81 mg) perfusate and both perfusate samples (1 ml) were analyzed for nicotine and cotinine by HPLC at regular intervals. About 18.6% of nicotine added to the maternal perfusate was transferred to the fetal side in 40 min and the maternal/fetal concentration ration reached 1. About 1% of nicotine was oxidized to cotinine in placenta and cotinine concentrations were equal at 100 min. When nicotine was added on the fetal side, part of it was metabolized to cotinine. However, the maximal concentration of cotinine was twice higher in fetal side than in maternal side. These observations suggest that accumulation of cotinine on fetal side may activate PG formation and trigger spontaneous abortions in pregnant smokers. (Supported by The Smokeless Tobacco Research Council and the Council for Tobacco Research, Inc.)

1615 DIFFERENTIAL LOSS OF SULFOTRANSFERASE (ST) GENE EXPRESSION IN RAT PRIMARY HEPATOCYTE CULTURES

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The feasibility of using rat primary hepatocyte cultures for studying ST has not been systematically examined. In the present study, ST mRNA levels in hepatocytes cultured in various conditions such as matrix (collagen, matrigel, collagen-collagen sandwich, or coculture with epithelial cells), medium (Waymouth's MB 752/1 and Modified Chee's Medium) and hormone supplement (0.1 μM dexamethasone, Dex) were examined by Northern blot analysis. ST1A1 mRNA levels decreased to about 50% of the initial mRNA level at 24 hr and to 20% at 96 hr in collagen, matrigel and coculture, with even less expression in the sandwich matrix. No difference in ST1A1 mRNA levels was observed between the two media in absence of Dex. Dex added to either medium resulted in ST1A1 mRNA levels greater than 100% of the initial mRNA level at 96 hr, with the greatest increase observed using the matrigel matrix and Chee's medium. The mRNA levels of other five STs fell to approximately 20% of their initial levels within 24 hr and less than 10% at 96 hr. Dex raised their mRNA levels to 20% of the initial levels at 96 hr. These data suggest that the ST1A1 mRNA level is better maintained in culture than the other STs analyzed. (Supported by NIH Grant ES-03192)

1616 CYSTEINE CONJUGATE β-LYASE-DEPENDENT BIOTRANSFORMATION OF THE CYSTEINE S-CONJUGATES OF THE SEVOFLURANE DEGRADATION PRODUCT COMPOUND A IN HUMAN, NONHUMAN PRIMATE, AND RAT RENAL TISSUE

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Fluoromethyl 2,2-difluoro-1-trifluoromethylvinyl ether (Compound A) is a fluorinated alkene formed by the degradation of sevoflurance in the anesthesia circuit. Compound A is nephrotoxic in rats and undergoes glutathione-dependent metabolism *in vivo*. Several nephrotoxic halogenated alkenes also undergo glutathione- and cysteine conjugate β-lyase-dependent biotransformation. These experiments were designed to test the hypothesis that cysteine *S*-conjugates of Compound A may undergo β-lyase-dependent biotransformation. *S*-(1,1,3,3,3-Pentafluoro-2-fluoromethoxypropyl)-L-cysteine, and *S*-[(1-fluoro-2-fluoromethoxy-2-trifluoromethyl)vinyl]-L-cysteine, the cysteine conjugates of Compound A, and *S*-(2-chloro-1,1,2-trifluoroethyl)-L-cysteine, the cysteine conjugate of chlorotrifluoroethylene, were incubated with kidney cytosol and mitochondria from rat, human and nonhuman primates (cynomolgus, rhesus, and marmoset), and β-lyase activity was determined by measuring pyruvate formation. The Compound A-derived cysteine conjugates and the cysteine conjugate of chlorotrifluoroethylene were substrates for cytosolic and mitochondrial β-lyase from human, nonhuman primate, and rat kidney. For all substrates, β-lyase activity was highest in the rat and lowest in the human and was higher in cytosol than in mitochondria. β-Lyase activity in human renal tissue was 8- to 30-fold lower than in rat renal tissue. The biotransformation of cysteine conjugates was inhibited by the β-lyase inhibitor (aminooxy)acetic acid and was stimulated by the amino group acceptor 2-keto-4-methylthiolbutanoic acid, indicating a role for β-lyase. These data confirm the presence of β-lyase activity in human and rat kidney and show that activity is also present in kidney tissue from nonhuman primates. (Supported by Abbott Laboratories and by NIEHS grant ESO3127)

1617 EFFECTS OF GLUCOCORTICOID AND DEHYDROEPIANDROSTERONE (DHEA) TREATMENT ON RAT HEPATIC SULFOTRANSFERASE GENE EXPRESSION

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Arylsulfotransferase IV (ASTIV) and hydroxysteroid sulfotransferase-a (HST-a) participate in xenobiotic detoxication and carcinogen activation. We previously reported that glucocorticoids increase HST-a and ASTIV mRNA levels in primary rat hepatocyte culture. In this study, the effects of *in vivo* treatment with glucocorticoids dexamethasone (DEX), betamethasone (BMET), hydrocortisone (HC), triamcinolone acetonide (TA), or adrenal steroid DHEA on rat hepatic ASTIV and HST-a gene expression were investigated. Male Sprague-Dawley rats (aged ~ 55 days) were treated daily for 3 days with vehicle or DEX, BMET, HC, TA or DHEA (100mg/kg ip in 2% Tween 20). Relative to vehicle treated controls, hepatic ASTIV mRNA levels were significantly increased (~209% and ~454%, respectively) in BMET and TA treated rats. DEX significantly increased HST-a mRNA levels (~50%), and BMET and DEX increased HST-a protein levels by ~ 2-and ~6-fold, respectively. Treatment with DHEA significantly suppressed HST-a mRNA levels (~54%) but changes in ASTIV mRNA were not significant. These results suggest that pharmacologic doses of glucocorticoid hormones positively regulate rat hepatic ASTIV and HST-a gene expression. Supported by NIEHS Grant ES05823

1618 NON-OXIDATIVE METABOLISM OF 2-BUTOXYETHANOL VIA FATTY ACID CONJUGATION IN FISHER 344 RATS

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2-Butoxyethanol (BE), a widely used industrial and household cleaning agent, causes acute hemolytic anemia in laboratory animals. One of the potential mechanisms of hemolytic anemia is conjugation of BE and its metabolites to membrane lipids resulting in disruption of the structural integrity of erythrocyte membrane. In our earlier study rat liver was found to contain highest radioactivity following [ethyl-1, 2-^{14}C]BE exposure. Therefore, fatty acid esters of BE were analyzed in the liver of male Fisher 344 rats following single oral administration of 500 mg/kg body weight [ethyl-1, 2-^{14}C]BE (70 μCi/kg). Animals were euthanized after 2 hr of treatment, hepatic lipids extracted and the neutral lipids separated using solid-phase extraction. Lipid esters, separated from neutral lipids by thin-layer chromatography, were subjected to reversed-phase HPLC. Approximately 85% of the ^{14}C-label present in the ester fraction was coeluted at retention times corresponding to fatty acid 2-butoxyethyl esters. Mass spectrometric analysis of the HPLC fractions corresponding to the esters showed molecular ion peaks and fragmentation patterns similar to 16:0, 18:0, 18:1, 18:2 and 20:4 fatty acid esters of BE, respectively. Formation of these esters was also catalyzed *in vitro* by the rat liver microsomal fatty acid ethyl ester synthase (FAEES) purified in our laboratory. Therefore, non-oxidative metabolism of BE appears to be similar to that of other xenobiotic alcohols and is catalyzed by FAEES.

1619 INHIBITION OF SULFATION OF THYROID HORMONES BY HYDROXYLATED POLYCHLOROBIPHENYLS

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Sulfation is one of the major conjugation reactions for drugs and environmental chemicals as well as for endogenous compounds. Sulfation is an important step in the inactivation of thyroid hormone, since thyroxine-sulfate (T4S) and 3,3', 5-triiodothyronine-sulfate (T3S) are preferred substrates for type

I iodothyronine deiodinase (ID-I). It has been suggested that sulfation of iodothyronines is an important pathway when ID-I is low, such is in non-thyroidal illness and during fetal development.

Recently, various halogenated phenols including pentachlorophenol, were found to be strong inhibitors of sulfotransferase (ST) activities towards thyroid hormone. In this study, 3,3',4,4'-tetrachlorobiphenyl (TCB) and his hydroxylated metabolites were tested in an *in vitro* assay for their effects on the sulfation of thyroid hormone.

ST activity was determined with 1 μM 3,3'-diiodothyronine (T2) and ca 80,000 cpm ^{125}I labelled hormone, 50 μM 3'-phosphophoadenosine-5'-phosphosulfate (PAPS) and 25 μg protein/ml rat cytosol in 200 μl 75 mM Tris-HCl, 1.5 mM EDTA (pH 7.2). Incubations were carried out at 37 °C for 30 min. The reaction was stopped on ice by adding 750 μl 0.1 M HCl, followed by Sephadex LH-20 chromatography.

It was shown that T2-ST activity was inhibited dose-dependently by hydroxylated metabolites to almost 100% but not by TCB itself. The inhibition potency was different and followed the order: 5-OH-TCB> 4-OH-TCB> 4,4'-$(OH)_2$-TCB> 2-OH-TCB. The IC_{50} for the 5-OH-metabolite was ± 0.63 μM.

These observations give reason to determine if PCB metabolites may affect fetal development by interfering with thyroid hormone sulfation.

1620 EFFECT OF UDP-GLUCURONOSYLTRANSFERASE (UDP-GT) INDUCERS ON 5'-DEIODINASE (DI) ACTIVITY

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UDP-GT inducers produce moderate to dramatic reductions in serum T_4 levels, whereas serum T_3 levels are reduced less. In addition to glucuronidation, deiodination (removal of I$^-$ from T_4, T_3, rT_3 or T_2) can potentially serve as a mechanism for reducing serum T_4 as well as maintaining serum T_3. This role of 5'DI would be supported if 5'DI (I & II) activity is increased in rats treated with UDP-GT inducers. Rats were fed diets containing various doses of the UDP-GT inducers: phenobarbital (PB), pregnenolone-16α-carbonitrile (PCN), 3-methylcholanthrene (3MC) or Aroclor 1254 (PCB) for 7 days. 5'DI-I and 5'DI-II activities were quantified by the release of ^{125}I$^-$ from ^{125}I-rT_3. Liver 5'DI-I activity was reduced by PB, PCN, 3MC & PCB treatments. Kidney 5'DI-I activity was reduced only by PCB. Thyroid 5'DI-I activity was only slightly increased by PCN and 3MC. Pituitary 5'DI-II activity was increased by only PCN and PCB. Brain 5'DI-II activity was increased by PCN, 3MC and PCB. In conclusion, 5'DI activity does not appear to be as important as glucuronidation in reducing serum T_4 or in maintaining serum T_3 because 5'DI activity is reduced, in liver and kidney, and increased in other tissues by only some of the UDP-GT inducer treatments. (Supported by NIH Grant ES-O7079).

1621 ROLE OF ESTERASE AND CYP2E1 IN URETHANE METABOLISM IN MURINE LUNG AND LIVER

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We investigated the role of esterase and CYP2E1 in the *in vitro* metabolism of urethane in murine lung and liver. Incubation of lung or liver microsomes with urethane resulted in 25% reduction in esterase-dependent *p*-nitrophenyl acetate hydrolysis. Incubation with the inhibitor, paraoxon (PX), reduced esterase activity by 85%, while incubation with phenylmethylsulfonylflouride (PMSF), an inhibitor of Hydrolase A, diminished enzyme activity by 40%. Immunoblotting with antiserum specific for Hydrolase A, showed diminished reactivity with lung or liver microsomes incubated with PX, PMSF, or urethane. Incubation of lung or liver microsomes with only PX or PMSF had no effect on CYP2E1-dependent *N*-nitrosodimethylamine demethylation, whereas incubation with urethane resulted in 25% reduction in enzyme activity. This loss of activity was exacerbated in lung (50%) or liver (65%) microsomes incubated previously with either PX or PMSF and subsequently with urethane. Inhibition of esterase activity prior to incubation with urethane produced diminished immunoreactivity of CYP2E1 with lung or liver microsomes. These results suggest that inhibition of Hydrolase A leads to increased bioactivation of urethane by CYP2E1. Supported by MRC of Canada.

1622 METABOLISM OF 1,1-DICHLOROETHYLENE BY CYP2E1 IN MURINE LUNG: SEXUAL DIMORPHISM IN FORMATION OF REACTIVE METABOLITES

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We investigated the cytochrome P450-dependent metabolism of 1,1-dichloroethylene (DCE) in murine lung microsomal incubations. The metabolites were identified as their GSH conjugates or hydrolyzed products, analyzed by HPLC and quantified with [^{14}C]-DCE. We compared the quantities of DCE-metabolites formed using microsomes from female and male mice. Also, the relative proportions of individual metabolites produced in lung were compared to those in liver. The DCE-epoxide was the major metabolite formed in both male and female lung and was trapped as two GSH conjugates, 2-(*S*-glutathionyl) acetyl glutathione [B] and 2-*S*-glutathionyl acetate [C]. Lower levels of the acetal of 2,2-dichloroacetaldehyde were also produced. Female microsomes yielded 2-fold higher levels of the DCE-epoxide conjugates and 1.6-fold greater production of the acetal than male microsomes. The ratio of acetal to epoxide in female lung microsomes (0.30) was significantly higher than in female liver microsomes (0.12). A CYP2E1-inhibitory antibody significantly reduced the production of the acetal and conjugates [B] and [C]. These data demonstrated that lung CYP2E1 metabolizes DCE to reactive intermediates of which the DCE-epoxide is the major metabolite. The extent of bioactivation is higher in female than in male mice, suggesting that the former may be more susceptible to DCE-mediated lung toxicity. Supported by MRC Canada.

1623 THE EFFECT OF DIETARY LIPID CONTENT AND COMPOSITION ON HEPTIC P450 ISOZYMES AND ESTRADIOL METABOLISM IN FEMALE RATS FED VIA A SYSTEM OF TOTAL ENTERAL NUTRITION (TEN)

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It has been suggested that elevations in dietary fat increase the risk of certain types of cancer. A potential mechanism whereby dietary fat might influence carcinogenesis is via increases in the metabolic activation of dietary and endogenous procarcinogens or decreases in carcinogen detoxication. Many of these reactions are catalyzed by the hepatic cytochrome P450-dependent microsomal monooxygenase system (HMO). In the current study, the effects of dietary fat content and composition have been examined on HMO in female rats. Groups of N = 6–8 female Sprague-Dawley rats (250 g) were fitted with enteral cannulae and fed for 21 days with defined liquid diets at a constant 160 kcal/kg$^{0.75}$/d. Experiments were conducted in which a dietary lipid content was altered from 5% to 40% total calories in which lipid composition was held constant. Alternatively, lipid content was held constant at 40% calories and lipid composition was altered from 92% saturated to 100% unsaturated fat. Both increasing fat calories and changing to unsaturated fats elevated expression of cytochrome P450 CYP 2E1 and associated p-nitrophenol hydroxylase activities in hepatic microsomes such that animals consuming 40% calories as unsaturated fat exibited a significant 2–3-fold elevation in CYP 2E1 activity and apoprotein ($p < 0.05$) over those animals consuming 5% saturated fat. By contrast whereas altering lipid composition had little or no effect, increasing lipid content of diets from 5% to 40% produced a significant ($p < 0.05$) decrease in CYP 1A2 and associated methoxyresorufin O-demethylase activity. When metabolism of 17β-estradiol was examined, the major product was estrone formed by 17β-hydroxysteroid dehydrogenase. The major hydroxylated products were 2 and 4 hydroxyestradiol and 2 and 4-hydroxyestrone. Small amounts of 16α- and 6-hydroxylated products were formed including measurable quantities of the genotoxic product 16α-hydroxyestrone. Increasing lipid content had no significant effects on hepatic estradiol metabolism, however altering composition to unsaturated fat resulted in significant ($p < 0.05$) elevations in the formation of 4-hydroxy and 6α-hydroxy products. Supported in part by CSRS 94–3434322–0353 USDA (T.M.B.).

1624 SELECTIVE EVALUATION OF RETINOIC ACID UDP-GLUCURONOSYLTRANSFERASE ISOENZYMES IN WISTAR AND GUNN RATS

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Hepatic microsomes contain a family of isoenzymes, UDP-glucuronosyltransferase (UDPGT, EC 2.4.1.17), which use UDP-glucuronic acid to conjugate a variety of substrates, including p-nitrophenol (pNP), chloramphenicol (CAP), bilirubin (BR), and retinoic acid (RA). Previously, we have reported the selective induction of these isoenzymes by 3-methylcholantherene (3-MC) phenobarbital (PB) and clofibrate (CLO) (FASEBJ.9(3): A168, No 979 and A169, No 986, 1995). All trans-RA and 13-cis RA UDPGT isoenzymes were induced by both 3-MC and CLO, but not PB. 13-cis RA UDPGT activity occurs at 3 times the all-trans RA UDPGT activity. Further characterization of the RA UDPGT isoenzymes was determined using Wistar and Gunn rat microsomes. Gunn rat, which presents a hereditary lack in BR UDPGT, indicated low activities for both pNP UDPGT (20%) and BR UDPGT (0–2%) relative to Wistar microsomal activities. 13-cis and all trans-RA UDPGT retained 20–30% of their activity. Induction by 3-MC or CLO in Gunn rats produced no change in RA UDPGT activities. This result is consistent with the previous one and suggests that a major portion of the RA UDPGT activity appears to be closely linked to the 3-MC inducible isoenzyme forms of UDPGT and the BR UDPGT. (Support provided by: ISU-CDFIN, USDA-CSRS 94–34115–0269.)

1625 *IN VIVO* AND *IN VITRO* METABOLISM AND TOXICITY OF ALLYL ACETATE IN MALE FISCHER 344 RATS

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Allyl acetate is widely used as an important intermediate in the synthesis of several industrial chemicals. The *in vitro* cytotoxicity and metabolism of allyl acetate were investigated in cultured liver slices obtained from male Fischer 344 rats and were compared to the *in vivo* toxicity. Various parameters of cytotoxicity were measured following incubation of cells with 0, 125, 250, 500, and 1000 μM allyl acetate. Dose- and time-dependent decreases in cellular ATP, potassium and protein synthesis accompanied the increase in lactate dehydrogenase (LDH) leakage. Similarly, oral administration of allyl acetate (60 mg/kg) resulted in an increase in plasma alanine aminotransferase (ALT) activity and periportal hepatocellular necrosis. *In vitro and in vivo* allyl acetate toxicity was blocked following pretreatment with triorthotolyl phosphate (TOTP), a carboxylesterase inhibitor, and pyrazole, an alcohol dehydrogenase inhibitor. Additionally, pyrazole protected against allyl alcohol toxicity in liver slices while TOTP pretreatment had no effect. HPLC analysis of *in vitro* samples revealed that [^{14}C]-allyl acetate was rapidly metabolized by the liver slices. Allyl alcohol was the predominant metabolite at the early time-points but it was converted to other metabolites during continued incubation. Consistent with the *in vivo* toxicity study, addition of TOTP to the liver slice incubation decreased the metabolism of allyl acetate, while pyrazole addition resulted in decreased metabolism of allyl alcohol, the hydrolytic metabolite of allyl acetate. The results suggest that the hepatotoxicity associated with allyl acetate is directly related to its metabolism to allyl alcohol, which is subsequently converted to acrolein. (Supported by the NIEHS, NO1-ES-35367 and Southwest Environmental Health Sciences Center Grant P30 ES06694-01).

1626 MODULATION OF DRUG METABOLISM DURING STRESS

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Previous experiments in our laboratory have shown that stress reduces hepatic monooxygenase activity in CD-1 mice. To determine the possible role of corticosterone (CORT) in modulating hepatic drug metabolism, adrenalectomized (ADX) and sham-operated (SO) mice were injected s.c. with CORT or exposed to a stress model (i.p. hypertonic saline; 10 ml/kg). Although hypertonic saline in SO animals increased serum corticosterone levels and decreased hepatic microsomal monooxygenase activity two hours after dosing, hepatic microsomal monooxygenase activity was not altered by CORT treatment in ADX or SO mice. In addition, corticosterone added to *in vitro* liver preparations did not alter cytochrome P-450-mediated activities. Since Kupffer cells (KC) have been implicated in the depression of drug-metabolizing enzymes following infection or tissue damage, the role of KC in stress-induced depression of monooxygenase activity was examined. Gadolinium chloride ($GdCl_3$), which selectively destroys KC, was administered i.v. to mice 24 hours before exposure to the stress model. Although stress-induced CORT increases at two hours were unaffected by $GdCl_3$ pretreatment, reductions in monooxygenase activity were prevented by $GdCl_3$ in a dose-dependent manner. Studies with selected soluble KC products (interleukins) showed that IL-1 and IL-6 are noncompetitive inhibitors of monooxygenase activity *in vitro* but no effect was seen *in vivo* within two hours of dosing. These results suggest that stress-induced adrenal product(s) other than corticosterone modulate liver KC activity which mediates hepatic monooxygenase activity by unknown factors.

1627 METABOLISM OF BROMODICHLOROACETATE IN MALE F344 RATS

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Bromodichloroacetate (BDCA) is a by-product of chlorination of drinking water which is a hepatocarcinogen. The replacement of a Cl by a Br greatly increases the metabolism of trihaloacetates which in the case of BDCA leads to high concentrations of dichloroacetate (DCA). Since DCA is also hepatocarcinogenic, it might play an important role in the effects of BDCA. Male F344 rats administered [^{14}C$_1$]-BDCA by gavage. The animals were housed individually in glass metabolism cages designed for separation of urine, feces and expired air. CO_2 production decreased and the amount of DCA eliminated in the urine significantly increased as the doses of BDCA increased. To investigate the effect of dose on the kinetics of BDCA and DCA, three groups of animals were dosed with 5, 20 and 100 mg/kg BDCA intravenously. Blood samples were analyzed for BDCA and DCA. The fractional elimination of BDCA was decreased with increasing dose ($t_{1/2}$ increased from 2 to 5 hr). In addition, the elimination of DCA produced from BDCA was even more slowly eliminated than BDCA ($t_{1/2}$ increased to 10.4 hr). Therefore, the toxicological effects of BDCA should resemble those induced directly by DCA. (Supported by NIEHS grant RO1-ES04648).

1628 CHARACTERIZATION OF URINARY METABOLITES FROM SPRAGUE-DAWLEY RATS AND B6C3F1 MICE EXPOSED TO [1,2,3,4-^{13}C]BUTADIENE

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1,3-Butadiene (BD) is used in the manufacture of plastics and polymers. Carcinogenic effects have been observed in laboratory animals exposed to BD, with mice being the more sensitive species. Metabolic oxidation of BD to epoxides is believed to be a crucial step in the initiation of tumors. Metabolites were characterized in urine of male Sprague-Dawley rats and B6C3F1 mice exposed to 800 ppm [1, 2, 3, 4-^{13}C]-butadiene for 5 h. *N*-acetyl-*S*-(2-hydroxy-3-butenyl)-L-cysteine, *N*-acetyl-*S*-(1-(hydroxymethyl)-2-propenyl)-L-cysteine, and *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine were present in rat and mouse urine, accounting for 88% and 73% of the total metabolites excreted, respectively. 3-Butene-1,2-diol was detected in rat (7%) and mouse urine (4%). Additional metabolites detected only in mouse urine that are derived from glutathione conjugation with epoxybutene were identified as *N*-acetyl-*S*-(1-hydroxy-3-butenyl)-L-cysteine (4%) and *S*-(1-(hydroxymethyl)-2-propenyl)-L-cysteine (5%). Mice also excreted *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine (5%) and *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine (4%), which could be derived from further metabolism of *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine. Mice excreted *N*-acetyl-*S*-(1-(hydroxymethyl)-2,3-dihydroxypropyl)-L-cysteine (5%), which could be derived from glutathione conjugation with BDE, while rats excreted 1,3-dihydroxypropanone (6%), which may be derived from hydrolysis of BDE. When normalized to body weight, the amount of BDE derived metabolites was four times greater in mouse urine than in rat urine. The greater toxicity of BD in mice compared with rats may be attributed to mice producing a larger amount of BDE-derived metabolites and rats having a greater ability to detoxify BDE via hydrolysis.

1629 *IN VITRO* TRANSESTERIFICATION OF COCAETHYLENE WITH ETHANOL: ETHYL ESTER EXCHANGE

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Co-administration of cocaine and ethanol produces the pharmacologically active metabolite, cocaethylene (ethylcocaine) as a result of transesterification. Our laboratory has recently shown that meperidine which contains a carboxyl ethylester group, undergoes ethyl ester exchange in the presence of ethanol. We hypothesized that cocaethylene will undergo a similar reaction. Homogenized liver (male Sprague-Dawley rats) in pH 7.4 phosphate buffer was centrifuged at 9,000 g and the resulting supernatant collected. Tubes containing liver supernatant, 50 uM cocaethylene, and 50 mM ethanol, ethanol-d_5, or control were incubated at 37° C for 4 hours. Samples were collected, extracted with a solid phase extraction system and assayed for cocaethylene and cocaethylene-d_5 via gas chromatography / mass spectrometry. Cocaethylene was converted to cocaethylene-d_5 in the presence of ethanol-d_5. A 4-fold increase in half-life and 3-fold increase in area under the concentration-time curve of cocaethylene was observed when ethanol was present compared to control.

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1630 ETHANOL AND DRUG METABOLISM: A BROAD CATEGORY OF METABOLIC INTERACTION VIA CARBOXYLESTERASE TRANSESTERIFICATION

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A microsomal carboxylesterase enzyme is responsible for the transesterification of cocaine to cocaethylene in the presence of ethanol. We hypothesized that other compounds with similar ester functional groups will undergo a reaction to form previously unreported ethyl ester metabolites and/or undergo ethyl exchange. Meperidine and methylphenidate, each containing a carboxyl ester group, were examined. Homogenized liver (Sprague-Dawley rats) in pH 7.4 phosphate buffer was centrifuged at 9,000 g. Liver supernatant, 50 uM drug, and 50 mM ethanol, ethanol-d_5, or control were incubated at 37° C. Samples were assayed for parent drug and ethyl ester formation products via gas chromatography/ mass spectrometry. Experiments were repeated in the presence of esterase inhibitors. Meperidine was converted to meperidine-d_5 in the presence of ethanol-d_5. This conversion was totally inhibited by non-specific and specific esterase inhibitors but was unaffected by physostigmine. A 10-fold increase in half-life (t 1/2) and 6-fold increase in area under the concentration-time curve (AUC) of meperidine was observed in the presence of ethanol. Ethylphenidate, a previously unreported metabolite, was formed in the presence of ethanol. This reaction was also totally blocked by esterase inhibitors but unaffected by physostigmine. No statistically significant difference in methylphenidate t 1/2 and AUC was seen with or without ethanol. Whole animal dosing is being performed and other candidate drugs likely to undergo this interaction are being examined. There are substantial clinical and toxicologic implications to this broad category of drug interaction with ethanol.

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1631 EFFECTS OF MOLYBDATE AND PENTACHLOROPHENOL ON THE SULFATION OF ACETAMINOPHEN

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Pentachlorophenol (PCP) is a competitive inhibitor of sulfotransferase (ST) and is used to ascertain the role of sulfation in toxicology. Recently, molybdate (Mo) also has been shown to inhibit the sulfation of various chemicals by decreasing the cosubstrate, 3'-phosphoadenosine 5'-phosphosulfate (PAPS) concentration. The purpose of this study was to compare the effectiveness of these two chemicals in inhibiting the sulfation of various doses of acetaminophen (AA) in the rat. PCP (40 μmol/kg) decreased the combined urinary and biliary excretion of AA-sulfate by 77,87,84 and 47% of the 0.1, 0.3, 1 and 3 mmol/kg doses of AA, respectively. Mo (7.5 mmol/kg) decreased the sulfation of these same doses by 49, 65, 62 and 82%, respectively. These data indicate that PCP is more effective in decreasing the sulfation of lower AA doses. This occurs most likely because at low AA doses there is less AA to compete with PCP for ST, thus PCP binds more ST. Conversely, Mo is more effective in decreasing sulfation of higher AA doses, most likely because the sulfation of higher AA doses require proportionately more available PAPS than lower AA doses, thus more sensitive to changes in PAPS. Therefore, PCP inhibits sulfation greatest at low AA doses when sulfation is limited by ST and Mo inhibits sulfation greatest at high AA doses when sulfation is limited by PAPS. (Supported by NIH Grant ES-03192)

1632 TISSUE SPECIFIC EXPRESSION OF SULFOTRANSFERASE ENZYMES IN THE RAT

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Sulfotransferases (STs) are a class of enzymes that catalyze the sulfonation of endogenous compounds as well as certain xenobiotics. The present study sought to examine the tissue distribution of certain sulfotransferase mRNAs including male-dominant STs (ST1A1, ST1C1 and ST1E2) and female-dominant STs (ST20–21, ST40–41 and ST60) by Northern-blot analysis with α-[^{32}P]dATP-labeled oligonucleotide probes specific for individual ST mRNAs. Tissue expression levels were quantified by phosphorautoradiographic analysis relative to liver expression of each ST. The following tissues were analyzed for ST expression: liver, brain, lung, heart, intestine, adrenal, kidney, spleen, pancreas, ovary, uterus, prostate and testes. Male-dominant ST1A1 expression was observed in liver, brain, lung, heart, adrenal, kidney, prostate and testes. ST1C1 expression was observed in liver, brain and kidney. ST1E2 expression was observed in liver and heart. Of the tissues analyzed, female-dominant ST 20–21 and 40–41 expression was observed only in liver. ST60 expression was observed in liver, brain, lung, intestine, kidney, ovary, and uterus. Extra-hepatic expression of ST mRNAs was only a small fraction of levels observed in liver except for ST1A1 which exhibited substantial expression in lung. These studies indicate variable tissue-specific expression of ST mRNAs with highest levels observed in liver. (Supported by NIH Grants ES-03192 and ES-07079)

1633 TRICHLOROETHYLENE METABOLISM BY HUMAN HEPATOCYTES IN PRIMARY CULTURE

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Trichloroethylene (TRI) contaminates groundwater and drives remediation costs. The cytochrome P-450 mediated metabolism of TRI yields chloral hydrate (CH), which is reduced to trichloroethanol (TCOH) and oxidized to trichloroacetic acid (TCA). Because the toxicity and hepatocarcinogenicity of TRI seem to be dependent upon metabolism, we sought to characterize the metabolism of TRI by human hepatocytes. TRI was exposed in headspace at concentrations of 25 to 10,000 ppm for up to two hours and samples were analyzed for CH, TCOH and TCA. TCOH was identified as the primary (95 + %) metabolite, and formation of TCOH was linear over 2 hours and was dose-dependent. CH was identified only at the early time points from the highest TRI concentrations. Results from acid-dependent extractions indicate TCOH glucuronidation. Preliminary data indicate little, if any, dichlorovinyl glutathione and no TCA were formed. These data support TCOH as the major TRI metabolite from human hepatocytes and agree well with the reported short half-life and metabolism of CH in the human. The human hepatocyte, as procured from organ donor agencies, may be well-suited to examine chemical metabolism in a system representative of whole-animal metabolism. Funded in part by the Strategic Environmental Research and Development Program (SERDP).

1634 COMPARISON OF THE *IN VITRO* METABOLISM OF TRICHLOROETHYLENE IN THREE SPECIES: RAT, MOUSE, AND HUMAN

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Trichloroethylene (TRI), a widely used industrial degreasing solvent, has become a common groundwater contaminant, thereby presenting the potential risk of human exposure. TRI is metabolized to chloral hydrate (CH), trichloroacetate (TCA), trichloroethanol (TCOH), and other compounds. Some rodents have developed hepatocellular carcinomas following exposure to TRI and/ or its metabolites. However, to date epidemiologists have been unable to demonstrate a link between known TRI exposure and toxicity in humans. Therefore, as part of an ongoing investigation into the toxicities associated with TRI exposure, the kinetics of TRI metabolism in the hepatic microsomes of three species were determined. CH and TCOH formation from TRI at pH 7.4 was evaluated by incubating rat, mouse and human hepatic microsomes with limiting concentrations of TRI at 37°C for 30 min. TRI was dissolved in acetone and spiked into the incubation mixture at 0.1% per final volume.

Although acetone is a known inhibitor of cytochrome P-450IIE1, the enzyme thought to be responsible for the conversion of TRI to CH, it was experimentally shown to have no effect on 2E1 activity at this level. The kinetics for microsomal TRI metabolism indicated K_m values of 55.5, 24.6 and 35.4 μM and V_{max} values of 1206, 360 and 1356 nmol/min/mg protein, respectively for the rat, human and mouse. Trichloroethanol was also formed from TRI in very small amounts. These data indicate that the kinetics of TRI metabolism may differ significantly depending upon the species evaluated, and care should be used when extrapolating rodent data for human risk assessment. Human in vitro studies like the ones conducted here are an invaluable step toward the accurate characterization of the biochemical pathways involved in human metabolism of TRI. Funded in part by the Strategic Environmental Research and Development Program (SERDP).

1635 DIETARY ASCORBIC ACID DOES NOT ALTER THE EXPRESSION OF THE 58-kDa ACETAMINOPHEN BINDING PROTEIN NOR THE ARYLATION OF OTHER CYTOSOLIC PROTEINS IN GUINEA PIG LIVER

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Ascorbic acid (AA) is known to alter the hepatotoxicity induced by Acetaminophen (APAP) overdose. When adult male guinea pigs (GP) were fed diets containing 0[O-AA], 0.1[L-AA], and 2.5%[H-AA] for four weeks, and liver proteins were separated on SDS-PAGE and stained with Coomassie blue, a marked decrease in the amount of a 60kDa protein was noted in the animals fed a diet devoid of AA. This was particularly prominent when the proteins were derivatized with monobromobimane and visualized by fluorescence. Since the major murine cytosolic protein that covalently binds APAP is of 58 kDa (58-ABP), studies were conducted to determine if varied AA intake may alter the levels of the 58-ABP and the profile of APAP-bound proteins in GP liver. Surprisingly, Western blot analysis with anti-58-ABP antibodies of cytosols from all groups failed to detect any 58-ABP, suggesting that this protein is not expressed in GP. Furthermore, when cytosols were cleared of glutathione (G-25 column), and incubated with APAP and a microsomal activating system from acetone-induced mouse liver, Western blot analysis with anti-APAP antibodies revealed no binding to a 58 kDa protein. However, APAP selectively arylated other prominent cytosolic target proteins with no apparent effect of varying the dietary content of AA. Hence, the protective effects of AA on APAP toxicity cannot be explained by diminished arylation of a 58-kDa target in the GP.

1636 BIOTRANSFORMATION OF ALL-*TRANS*-RETINAL TO ALL-TRANS-RETINOIC ACID IN RAT CONCEPTAL CYTOSOL AND MICROSOMES

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Enzymatic catalysis of the oxidation of all-trans-retinal (t-retinal) to teratogenic all-trans-retinoic acid (t-RA) was demonstrated in 105,000 g × 1.5 hr supernatant (cytosolic) and particulate (microsomal) fractions of rat conceptal homogenates at day 12 of gestation. Products of the oxidation were identified with high performance liquid chromatography (HPLC) by comparing elution times with those of authentic standard retinoids. Under the experimental conditions utilized, the primary oxidation product of t-retinal was t-RA with only trace amounts of 13-cis-retinoic acid (13-cis-RA) and 9-cis-retinoic acid (9-cis-RA) and no other metabolites detectable. For both cytosolic and microsomal-catalyzed oxidations of t-retinal, NAD appeared to be the most effective cofactor in facilitating the generation of t-RA, suggesting that NAD-dependent, cytosolic/microsomal retinal dehydrogenase(s) were the primary enzymes participating in the oxidation of t-retinal. Under the same experimental conditions, the NAD-dependent cytosolic retinal dehydrogenase(s) exhibited approximately ten-fold higher specific activities than microsomal dehydrogenase(s), indicating that the cytosolic fraction of rat conceptal homogenates is a major site for the biotransformation of t-retinal to t-RA. Various enzyme inhibitors were used to further identify the NAD-dependent cytosolic/microsomal dehydrogenase(s). At up to 10 mM concentrations, 4-methyl pyrazole (inhibitor of class IADH), ethanol (competitive inhibitor of class IADH), and 3-amino-1,2,4-triazole (inhibitor of catalase), showed no significant inhibitory effects on the oxidation of t-retinal. By contrast, low concentrations of citral (3,7-dimethyl-2,6-octadienal, 0.1 mM) strongly inhibited the biotransformation of t-retinal to t-RA catalyzed by either NAD-dependent cytosolic or microsomal dehydrogenases.

1637 MODULATION OF DRUG METABOLIZING ENZYMES IN GUINEA PIG LIVER BY ASCORBIC ACID

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Ascorbic acid (AA) has been shown to play a significant role in the metabolism of xenobiotics and carcinogens. However, studies up to the present have confined themslves to cytochrome P-450. The present study was designed to determine if varied AA intakes have any effects on the Phase-I (Ph-I) and Phase-II (Ph-II) drug metabolizing enzymes (DMEs) in guinea pigs (GP). Ph-I components are those related to metabolism of xenobiotics. They include cytochrome P-450 and mixed function oxygenase activity. Ph-II components are those concerned with conjugation and detoxification. They include, glutathione S-transferase (GST), UDP-glucuronyl transferase (UDP-GT), DT-diaphorase (DTD), glutathione (GSH), etc. The GP were chosen as an animal model as they are very similiar to humans in lacking the *de novo* synthesis of AA. Adult male Hartley GP weighing 350-400g were fed a diet containing 0[O-AA], 0.1[L-AA] and 2.5% [H-AA] AA for four weeks. The food consumption and weight gain were not significantly different in different groups. Liver microsomes and cytosols were isolated and Ph-I and Ph-II DMEs were determined. 0-AA group showed a significant decrease in Ph-I DMEs-cytochrome P-450, cytochrome b_5, NADPH cytochrome c reductase, aminopyrine N-demethylase and total microsomal heme as compared to H-AA and L-AA groups. 0-AA group also showed low activity of Ph-II DMEs (GST, UDP-GT, DTD and GSH). Ph-I and -II DMEs showed a positive correlation. This observation is of great significance because it implies that adequacy of AA as indicated by L-AA and H-AA would considerably improve the efficiency of the body in counteracting the harmful effects of xenobiotics, possibly including carcinogens. The ability of AA to positively influence both activating (Ph-I) and detoxifying (Ph-II) enzymes reveals a new facet of the beneficial effects of AA.

1638 PHARMACOKINETICS AND METABOLISM OF VINYL FLUORIDE (VF) IN VIVO AND IN VITRO

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VF is an inhalation carcinogen in both rats and mice. We showed previously that mice metabolize VF ~3x faster than rats (*The International Toxicologist*, 1995, 63-PF-5). In this study the pharmacokinetics of VF was investigated *in vitro*. Microsomes were isolated from rat and mouse liver by differential centrifugation, and incubated in a sealed vial with VF and an NADPH regenerating system. Headspace concentrations of VF (10 – 300 ppm) were monitored over time by GC. Consistent with the *in vivo* data, VF was metabolized faster by mice microsomes than by rat microsomes (V_{max} = 2.7 nmol/hr/mg protein vs. 0.8 nmol/hr/mg protein, respectively). The rates of metabolism by human liver microsomes were in the same range as those found with rodent liver microsomes (V_{max} = 0.4 – 2.5 nmol/hr/mg protein) with one sample being similar to mice and the others similar to rats. The K_m for all three species was essentially the same (0.5 μM). Metabolism rates in human microsomes were correlated with the amount of CYP 2E1 (the main P-450 isozyme responsible for the oxidation of small organics) by Western blotting. A very strong correlation was found between rate of metabolism and CYP 2E1 expression levels. Additional data from a specific functional assay for CYP 2E1 (chlorzoxazone 6-hydroxylation) supported this correlation. It is concluded that interindividual variability in the expression of CYP 2E1 may be a key factor for the determination of VF pharmacokinetics and metabolism in humans. Furthermore, the excellent correspondence between *in vivo* and *in vitro* kinetics in rodents increases substantially the degree of confidence for human *in vivo* predictions.

1639 ROLE OF CYP2E1 AND CYP2A6 IN THE HYDROXYLATION OF 4-NITROPHENOL BY HUMAN LIVER MICROSOMES

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The hydroxylation of 4-nitrophenol (4-NP) to 4-nitrocatechol by a bank of human liver microsomes was studied over a wide range of substrate concentrations. At low substrate concentrations (<100 μM), the sample-to-sample variation in 4-NP hydroxylase activity correlated well with chlorzoxazone 6-hydroxylase activity (r^2 = 0.90), which confirms previous reports

that 4-NP hydroxylation is a marker of CYP2E1. Furthermore, of seven cDNA-expressed human P450 examined, only CYP2E1 catalyzed appreciable rates of 4-NP hydroxylation at low substrate concentrations (100 μM). At higher substrate concentrations (pilt500 μM), 4-NP hydroxylase activity correlated poorly with CYP2E1 (r^2 = 0.41). Furthermore, the rates of 4-NP hydroxylation at low (30 μM) and high (3000 μM) substrate concentrations did not correlate well with each other (r^2 = 0.56), suggesting that a second enzyme catalyzes the 4-hydroxylation of 4-NP at high substrate concentrations. At a high substrate concentration (1500 μM), four cDNA-expressed enzymes other than CYP2E1 catalyzed appreciable rates of 4-NP hydroxylation; namely CYP1A2, CYP2A6, CYP2C9 and CYP3A4. Inhibitors of CYP1A2 (furafylline), CYP3A4 (troleandomycin and ethynylestradiol) and CYP2C9 (sulfaphenazole) did not inhibit the hydroxylation of 4-NP by human liver microsomes. In contrast, the CYP2A6 inhibitor, 8-methoxypsoralen, inhibited the hydroxylation of 4-NP by human liver microsomes, and the degree of inhibition was dependent on the levels of CYP2A6. Furthermore, the difference between the rates of 4-NP at low (30 μM) and high (3,000 μM) substrate concentrations was highly correlated (r^2 = 0.86) with coumarin 7-hydroxylase activity. These results suggest that the second enzyme involved in the hydroxylation of 4-NP is primarily CYP2A6. Supported by NIH grants ES03765 and ES07079.

1640 CYTOCHROME P-450 DEPENDENT TESTOSTERONE METABOLISM BY CULURED RAT AND MOUSE LIVER SLICES

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Steroid hormones are useful as substrates for distinguishing multiple cytochrome P-450 isoforms due to their unique metabolite profile. Hepatic cytochrome P450s catalyze hydroxylation of the steroid D ring at various positions and the corresponding metabolites can be detected by HPLC. Precision cut liver slices from Fischer 344 male rats and C57B1/10ScN male mice were cultured in serum free Chee's essential medium supplemented with insulin, transferrin, selenium, dexamethasone and epidermal growth factor. Slices were oxygenated with 95% O_2 and incubated at 37°C using dynamic a roller system. Cytochrome P-450 activities were induced with 10 μM βNF or 1 mM phenobarbital. 100 nM of 17-β testosterone was added at 72 hrs to uninduced and induced slices. At 96 hrs, the media were harvested, deconjugated and extracted with ethyl acetate. The metabolites were seperated on a LC-18 column equilibrated with 15% acetonitrile, 20% methanol, 0.1% acetic acid and eluted using a linear gradient of 95% acetonitrile. Liver slices metabolized testosterone by hydroxylation at 15β (CYP3A1), 6β (1A1, 3A4), 7α (2A1), 16α (2B1, 2C2) positions and oxidation at the 17α (2B1, 2C2) position. Liver histology remained mostly normal during culture. This analysis provides a qualitative and quantitative method of assessing enzymatic function of several cytochrome P-450s. Supported by The Johns Hopkins Center for Alternatives to Animal Testing.

1641 ISOFORM-SPECIFIC CYTOCHROME P450-MEDIATED OXIDATION OF HYDROQUINONE

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Although hydroquinone (HQ) undergoes microsomal NADPH-dependant oxidation, the role of specific isoforms of cytochrome P450 (CYP) in this process is not known. We therefore determined CYP-catalyzed HQ oxidation by trapping 1, 4-benzoquinone (BQ) with N-acetylcysteine. CYP catalyzed HQ oxidation was 3.6-fold higher in rat hepatic microsomes than in renal microsomes. Hepatic CYP-catalyzed HQ oxidation was highest in Fischer 344 rats = Sprague-Dawley rats = BALB/c mice = B6C3F1 mice > Syrian hamsters > guinea pigs. Rat hepatic HQ oxidation was inhibited 42, 71, 33 and 73% by 1.0 mM diethyldithiocarbamate (DC), 20 mM aminobenzotriazole (ABT), 20 mM cimetidine (CI), and DC plus CI, respectively. Renal microsomal HQ oxidation was inhibited 78, 79 and 0% by DC (at 0.1 mM), ABT, and CI respectively. HQ oxidation was also determined in microsomes obtained from a human B lymphoblastoid cell line expressing various human CYP's. HQ oxidation was 9.3 ± 3.7, 8.3 ± 1.1, 2.3 ± 0.2, 0.4 ± 0.2 and 0 pmol/pmolCYP/min in microsomes prepared from CYP1A1, 2E1, 3A4, 1A2 and 2B6 expressing cells, respectively. The results indicate species and isoform specific differences in HQ oxidation. Moreover, humans are capable of catalyzing the CYP-mediated oxidation of HQ to BQ, and hepatic CYP's 2E1 and 3A1/2, and renal CYP2E1 are the predominant CYP's catalyzing HQ oxidation. It is of interest that CYP1A1, which has low activity in human liver, but is strongly inducible in extrahepatic tissues, such as lymphocytes, placenta and lung, possesses high HQ oxidase activity. (GM39338).

1642 POSITIVE IDENTIFICATION OF PREGNENOLONE 16 α CARBONITRILE AND DEXAMETHASONE INDUCED GENE BY DIFFERENTIAL DISPLAY TECHNIQUE- RAT ADRENOMEDULLIN PRECURSOR GENE

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Dexamethasone (DEX) is a steroid hormone having a wide-spread effects on carbohydrate, lipid and protein metabolism and has shown an inhibitory effect on the immune system (inhibits Il-8 and Il-2 production, Mukaida W. et. al. and Vacca A. et. al. respectively). DEX treatment of rats or humans increases the amount of cytochrome P450 of the 3A family in the liver. To evaluate the complete profile of genes induced by DEX or PCN (an anti-steroid agent) on gene induction and inhibition, Sprague-Dawley female rats were treated with DEX or PCN at 85mg/kg. Differential Display (mRNA RT-PCR) was employed in the isolation and the identification of cDNA fragments affected by DEX and/or PCN. cDNA fragments were identified using slight modifications to the previous described technique of Liang, et. al. A total of 57 DEX, 29 PCN induced, 12 DEX and 7 PCN inhibited cDNA fragments were identified and isolated by this technique. We have identified six true positives cDNA fragments. The size of these fragments have been estimated between 2–7 hundred base pairs. They were identified on Northern blots using randomly labeled probes. Probes were reamplified using modified primers as described by Wang, C. and Feuerstein, G. 1995. After reamplification, the resulting cDNA bands were isolated by electrophoresis on 6% acrylamide gel. The purified material was used for sequencing employing Sanger dideoxy method with the aid of Sequenase PCR product sequencing kit. No significant homology was found in Genbank for three of these cDNA fragments. One of the amplified cDNA showed strong homology (>90%) with a previously reported RAT Adrenomedullin precursor gene. While Northern blot analysis has reportedly shown adrenomedullin transcript in the adrenal gland, lung, kidney, heart and spleen, but very little was detected in liver indicating that is not constitutively expressed. However consistent with our strategy for isolating such genes, we found adrenomedullin is inducible by DEX and PCN in female rat liver. This approach has great promise as a simple means for elucidating the genetic basis of the functional importance of the liver in responding to xenobiotics. (Supported by National Institutes of Health Grants ES05744 and ES04238).

1643 INTOXICATION DUE TO INGESTION OF BITTER ALMOND OIL CONTAMINATED WITH NITROBENZENE: CLINICAL EXPERIENCE

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Between April and July of 1993 were reported nationwide, 9 cases of intoxication due to the ingestion of bitter almond oil, involving 13 patients: 7 adults and 6 children. Initially, it was considered an intoxication with cyanide, because almond, either bitter o sweet, posses in their composition a substance know as "Amigdaline", a cianogenic glycoside. By itself it is not toxic, however, by enzimatic hydrolysis it can liberate cyanide and produce toxicity. Qualitative determinatios of cyanide were developed for two of the patients, obtaining (+) result, confirming the initial diagnosis. The most frecuent clinical manifestations were: vomit, dizzines, oral, distal or general cianosis, respiratory depression, convulsions and generalized weakness. The seriouness of the cases required the patients to be hospitalized inmediately in the ICU of the different medical centers. Having once evaluated the cases, attention was called to the fact most patients, in addition to presenting the described clinical manifestations, were also presentiong a very pronounced cianosis, anemia, haemolisis and high levels of Met Hgb; symptoms that did not agree completely with the clinical manifestations that characterize any our initial diagnostic impressions, guidind us to a "contaminating" toxic agent of the oil, able to produce Met Hgb. A very exhaustive bibliographical research was conducted, also, there were developed different qualitative tests and analyses for the almond oil samples, obtaining as a result (+) presence of nitrobenzene. This result was confirmed later, by IR and RMN.

1644 EFFECT OF SOIL ON THE GENOTOXICITY OF MANUFACTURED GAS PLANT RESIDUE

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The modulating effect of soil on the bioavailability of xenobiotics such as chlorinated hydrocarbons has been extensively reported. However, limited studies have investigated the effect of soil on the bioavailability of polycyclic aromatic hydrocarbons (PAH). Therefore, the following study was conducted to evaluate the potential of soil to modulate the bioavailability of the genotoxic chemical components of manufactured gas plant residue (MGP). The genotoxicity of neat MGP, soil contaminated with MGP, and organic extracts of soil contaminated with MGP was evaluated. Soil samples were incorporated into a rodent powder diet (20 g soil/100g diet) while neat MGP and organic extracts of soil were incorporated into a basal gel diet at levels comparable to the PAH content of soil samples. Groups of mice were fed diets for a total of 15 days. Genotoxicity was evaluated by measuring chemical DNA adduct formation in lung tissue using the ^{32}P-postlabeling technique. The level of chemical DNA adducts were highest in animals ingesting neat MGP and organic extracts of soil contaminated with MGP (0.16 – 3.1 pmol adduct/mg DNA). In contrast, adduct levels were significantly lower in animals ingesting soil contaminated with MGP. In these animals, adduct levels could only accounted for 7 – 17% of the levels observed in animals ingesting neat MGP or organic extracts. The observed reduction in chemical DNA adduct formation suggests that the genotoxic components of MGP are less bioavailable when MGP is associated with soil. (Supported by EPRI RP-2963)

1645 ACTIVATION OF BENZO[C] PHENANTHRENE TO DNA-BINDING METABOLITES DIFFERS IN HUMAN MAMMARY MCF-7 CELLS AND MOUSE SKIN

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Benzo(*c*)phenanthrene (BcPh) is an environmental contaminant with low carcinogenic activity in rodent bioassays. BcPh-3, 4-diol-1, 2-epoxides (BcPhDE), however, are among the most tumorigenic diol epoxides known. To determine whether BcPh can be bioactivated to form DNA-binding metabolites in human cells, MCF-7 cells were treated with 10μM BcPh or 1μM BcPh-3, 4-diol for 48 hours. The BcPh-DNA adducts were ^{32}P-postlabeled and analyzed by reversed-phase HPLC. The major BcPh adducts in both treatments were identified as two (+)BcPhDE-1 (*S,R,S,R*)dA adducts, a (-)BcPhDE-2(*R,S,S,R*)dA adduct and a (-)BcPhDE-2(*R,S,S,R*)dG adduct. The majority of the adducts formed were dA adducts in both BcPh and BcPh-3, 4-diol treated cells. The total levels of binding were 3.1 and 6.3 pmol/mg DNA in BcPh and BcPh-3, 4-diol-treated cells, respectively. In BcPh-treated cells, (-)BcPhDE-2dA was the adduct present in the greatest amount after 72 hours. To determine whether BcPh is metabolically activated by similar pathways in mouse skin, female SENCAR mice were treated topically with 2μmol BcPh for 48 hours or 0.4μmol BcPh-3, 4-diol for 24 hours. In BcPh-treated mice, 3 small BcPhDE-DNA adducts were detected (0.7pmol/mg DNA), however, the 3 major unidentified adducts present (7.5pmol/mg DNA) were not formed from this diol epoxide. In contrast, BcPh-3, 4-diol-treated mice formed only BcPhDE-DNA adducts. The low formation of BcPhDE in mouse skin may explain the low tumorigenicity of BcPh in this tissue. These results indicate that bioassays in mouse skin may not provide an appropriate assessment of the potential carcinogenic risk BcPh represents to humans. Supported in part by CA40228(WMB) and CA17613(SA).

1646 EVALUATION OF THE POTENTIAL ROLE OF AROMATIC HYDROCARBON (AH) RECEPTORS IN CALCIUM SIGNAL-ING BY POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN HUMAN T CELLS

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Alterations in Ca^{2+} signaling have been associated with the immunotoxicity of PAHs, and perhaps certain halogenated aromatic hydrocarbons (HAHs), such as 2, 3, 7, 8-dibenzo-*p*-dioxin (TCDD). PAHs and HAHs are known to induce CYP1A1 by interaction with cytosolic Ah receptors (AhR). The purpose of the present studies was to determine whether AhR are involved in Ca^{2+} signaling produced by PAHs and HAHs in human T cells. Human peripheral blood T cells were obtained following Ficoll-Hypaque density gradient sedimentation and nylon wool fractionation. Intracellular Ca^{2+} was measured by flow cytometry. Results indicated that 10 μM 7, 12-dimethylbenz(a)anthracene (DMBA) and benzo(a)pyrene (BaP) produced a rapid (30 min) and a sustained (18 hr) increase in Ca^{2+} in human T cells, whereas TCDD (100 nM) produced an increase in Ca^{2+} at 30 min only. The increase in Ca^{2+} produced by DMBA and BaP was found to be partially blocked by 10 μM α-naphthoflavone (ANF). Since ANF is both an AhR antagonist and a cytochrome P450 inhibitor, we looked for AhR mRNA by RT-PCR in human T cells. We found extremely low levels of detectable AhR message in CD5 sorted human T cells, compared to non-CD5 positive cells. The lack of detectable AhR mRNA in human T cells suggests that AhR may not be involved in Ca^{2+} signaling produced by PAHs and HAHs. Supported by NIH ES05495, ES06273, & P30 ES06096

1647 INDOOR PAH AND CARBON MONOXIDE POLLUTION IN TRADITIONAL HOUSES IN BURUNDI

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Wood combustion is used as major energy source in African countries and could result in health treathening indoor pollution problems. Standard methods were used to determine indoor concentration of particulate and gaseous polycyclic aromatic hydrocarbons (PAH) and passive dosimetry was used to measure carbon monoxide in traditional houses in Burundi. The urinary excretion of 1-hydroxypyrene (1-OHP) was measured in occupants of these houses and compared with that of people living in the capital Bujumbura. Mean concentrations of naphtalene, fluorene, phenanthrene and acenaphthene exceeded 1 μg/m^3 and that of benzo(a)pyrene was 0.07 μg/m^3. Two- to 4-ring PAH were mainly present in the gas phase while 5- and 6-ring PAH appeared principally in the solid phase. Carbon monoxide mean concentration was 42 mg/m^3 and correlated with total PAH concentration ($r = 0.70$, $p < 0.001$). Mean urinary 1-OHP in people living in traditional houses (1.50 μmol/mol creatinine) was much higher than that of people living in the capital (0.05 μmol/mol creatinine) but was correlated neither with total PAH nor with pyrene air concentrations. The elevated concentrations of the studied contaminants constitute potential health hazards for the population. Supported in part by the Canadian International Development Agency.

1648 PURIFICATION AND CHARACTERIZATION OF HEPATIC AND INTESTINAL PHENOL SULFOTRANSFERASE WITH HIGH AFFINITY FOR BENZO(A)PYRENE PHENOLS FROM CHANNEL CATFISH

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Studies have shown that several phase 2 enzymes, including phenol sulfotransferase (PST) with 3-, 7-, or 9- hydroxybenzo(a) pyrene (OHBaP) have high activity in catfish intestinal mucosa. PST was purified from catfish hepatic and intestinal cytosol using DEAE-Sepharose CL-6B ion exchange chromatography and ATP-agarose affinity chromatography. Hepatic and intestinal PST activity toward 9-OHBaP was purified 344 and 286-fold, respectively, as compared to cytosol from liver and intestinal mucosa. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that one band with a molecular weight of about 41,000 was purified from intestine, while two bands of about 41,000 and 31,000 were obtained from liver. Antibodies raised against human P-PST (phenol-sulfating PST, courtesy of C.N. Falany) cross-reacted strongly with the 41,000 molecular weight peptides from liver and intestine, but weakly with the 31,000 molecular weight peptide of liver. K_m values for 9-OHBaP were 36 nM and 41 nM for purified hepatic and intestinal enzyme respectively. The V_{max} values were 223 nmol/min/mg and 286 nmol/min/mg protein for purified hepatic and intestinal enzyme respectively. At 9-OHBaP concentrations > 1 μM, substrate inhibition was observed. The apparent K_m values for the cofactor PAPS were 0.17 μM and 0.18 μM for purified hepatic and intestinal PST respectively. Attempts to obtain N-terminal sequence analysis of each of the pure PST proteins were unsuccessful, due to blocking. The amino acid compositions of each peptide were obtained and showed that the 31,000 peptide could not be a part of the 41,000 peptide. Supported by US PHS, ES O5781.

1649 DEPRESSION OF LOCOMOTOR ACTIVITY IN RATS AFTER ACUTE EXPOSURE TO BENZO(A)PYRENE BAP)

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The effect of acute doses of BaP on motor activity were studied in male and female F-344 rats. The rats were dosed at 0, 100, 300, and 600 mg/kg by oral gavage and spontaneous motor activity was measured for 24 hrs in Digiscan Activity Monitors. BaP induced a significant dose dependent decrease in locomotor activity (30–55%). Stereotypic behavior was also significantly decreased (30–60%) in all BaP treated groups in a dose dependent manner, and differences were most significant during the nocturnal phase of the rat cycle between 4–8 hrs after BaP exposure. No significant differences between control and treated animals were observed 12 to 24 hrs after B(a)P exposure and no significant sex differences were detected. These data indicate that single acute doses of BaP can impair locomotor activity up to 12 hrs after exposure and suggest that locomotor activity may serve as a sensitive indicator of neurotoxicity.

1650 ACTIVATION OF AH RECEPTOR-DEPENDENT GENE EXPRESSION IN THE ABSENCE OF EXOGENOUS LIGAND

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The aromatic hydrocarbon receptor (Ah receptor) is a ligand-activated transcription factor that plays an essential role in induction of polycyclic aromatic hydrocarbon (PAH) metabolizing enzymes. The Ah receptor is also believed to have other important physiological functions, such as regulation of cell proliferation, differentiation and apoptosis. Activation of the Ah receptor by exogenous compounds such as the environmental pollutants benzo(a)pyrene and TCDD, and by several naturally occurring heterocyclic compounds, has been well documented; however, endogenous mechanisms for Ah receptor activation are unknown. We have explored AhR-dependent gene expression in the absence of added exogenous ligand by transfection of plasmids encoding the AhR cDNA into different cell lines. In the monkey kidney epithelial cell line CV-1, we find that exogenous ligand is not needed to achieve a high expression level of reporter constructs containing AhR responsive elements. Reporter gene expression levels are dependent on the presence of the Ah receptor nuclear translocator protein (ARNT) and are 5- to 10-fold higher than those obtained in parallel transfection experiments in the mouse hepatoma cell line, Hepa-1. These results suggest that there is an endogenous mechanism for activation of the Ah receptor that is independent of exogenous ligand and that this mechanism is highly elevated in CV-1 cells. *[Supported by NIH Grants R01 ES06273 and P30 ES06096]*

1651 DETECTION OF PYRENE METABOLITES IN URINE OF HUMANS OCCUPATIONALLY EXPOSED TO PAHs

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Human exposure to polycyclic aromatic hydrocarbons (PAHs) is often assessed by quantitating 1-hydroxypyrene in urine. However, the method employed for this analysis requires an overnight enzyme hydrolysis step which may be incomplete. We have recently developed a new method that does not require hydrolysis and is capable of detecting glucuronic acid and sulfate conjugates of pyrene as well as free 1-hydroxypyrene in a single analysis. This method involves solid phase extraction of urine followed by separation using HPLC equipped with a phenyl modified reverse phase column and a fluorescence detector. This method was used to quantitate pyrene excretion in urine obtained from 44 individuals working in an operational manufactured gas plant in the Czech Republic. 1-Hydroxypyrene glucuronide conjugate was the major metabolite detected with levels ranging from 0.04 – 0.94 μg/g creatinine. The levels of sulfate conjugate and 1-hydroxypyrene were much lower than the glucuronide conjugate. Levels of these metabolites ranged from 0.002 – 0.41 μg/g of creatinine. These results indicate that the new method is suitable for detecting pyrene metabolites in urine from humans occupationally exposed to PAHs. The excretion levels of pyrene metabolites will be compared to exposure histories of individuals. (Supported by EPRI RP 2963).

1652 FLUORANTHENE MEDIATED APOPTOSIS IN MURINE T CELL HYBRIDOMAS IS AH RECEPTOR INDEPENDENT

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Recent studies suggest that environmental chemicals such as polycyclic aromatic hydrocarbons (PAH) compromise the immune system in part through programmed cell death (apoptosis). Nevertheless, mechanisms through which PAH induce apoptosis remain elusive. In particular, the role of the 8S AhR remains controversial. To extend our previous studies to the T cell compartment and to develop a clonal system in which intracellular signals leading to PAH-induced apoptosis can be dissected, the ability of fluoranthene, a ubiquitous, but less-well studied PAH, to induce apoptosis in murine T cell hybridomas was evaluated. Particular emphasis was placed on the role of the 8S AhR. The data indicate that: 1) three of four hybridomas studied undergo apoptosis within 8 hours of fluoranthene exposure; 2) fluoranthene induces growth arrest concurrent with apoptosis; 3) at doses sufficient to induce lymphocyte apoptosis, fluoranthene does not induce AhR nuclear translocation in cells expressing high AhR levels; 4) fluoranthene-responsive hybridomas do not express AhR mRNA or protein; 5) the $Ca^{2}+$ chelating agent EGTA partially inhibits fluoranthene-induced apoptosis. It is concluded that: 1) fluoranthene is a potential immunosuppressant; 2) apoptosis plays an important role in PAH immunotoxicity; 3) fluoranthene-mediated T cell death and growth arrest are AhR independent; 4) there are significant similarities between PAH- and antigen-specific receptor-mediated apoptosis. These findings encourage consideration of AhR-independent events in PAH risk assessment. Supported by RO1-ES06086.

1653 THE POLYCHLORINATED BIPHENYL (PCB) MIXTURES AROCLOR 1242 (A1242), AROCLOR 1248 (A1248) AND AROCLOR 1254 (A1254) STIMULATE CONTRACTIONS OF DAY 10 PREGNANT RAT UTERI

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PCBs reduce the gestation age of women and nonhuman primates. Aroclors are technical mixtures of PCBs and constitute the major forms of industrial PCBs. In this study we hypothesized that shortened gestation length by PCBs was mediated by stimulation of uterine oscillatory contraction. Longitudinal uterine strips from midgestation rats were mounted in standard muscle baths interfaced with a polygraph. Isometric contractions were recorded during exposure to A1242 (3 to 100 μM), A1248 (3 to 300 μM), and A1254 (3 μM to 1mM) added cumulatively at 20 min intervals. As a solvent control, equal volumes of dimethyl sulfoxide (DMSO) were used. The frequency of contraction increased in a dose-dependent manner with all three Aroclors. The ED_{50} of A1242, A1248, and A1254 was 53 μM, 121 μM, and 151 μM, respectively. Even after removal of Aroclors, the frequency of contraction remained stimulated for at least 4 h. In order to determine the relationship between exposure time and uterine contraction, three doses of A1242 were selected (10, 50, and 100 μM) and uterine strips were exposed to each concentration for 60 min. The frequency of uterine contractions increased as the time of exposure increased until 60 min, at which time maximum stimulation was shown. These data show that the stimulation of uterine contraction depends on the dose and the duration of exposure, and that the response is not readily reversible. Also, because the apparent Aroclor potency for stimulation of uterine contraction was A1242 > A1248 > A1254, the more lightly chlorinated PCBs may have more prominent stimulatory effects on rat uterine oscillatory contraction.

1654 INHIBITION OF SERCA-TYPE CALCIUM ATP-ASES BY POLYCYCLIC AROMATIC HYDROCARBONS: INITIAL EVIDENCE THAT INHIBITION MAY NOT BE VIA DIRECT MECHANISMS

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Studies by Krieger *et al, [Toxicol. Appl. Pharmacol.* **133**, 102-108, 1995] have shown that PAHs that produce a sustained increase in Ca2+ in human T cells also inhibit calcium ATP-ase pumps (SERCA) present in the endoplasmic reticulum (ER), which are responsible for Ca2+ reuptake into ER storage pools after Ca2+ mobilization into the cytoplasm. The purpose of the present studies was to examine the direct effects of PAHs on cloned SERCA enzymes. Full length clones encoding rat SERCA 1, 2A, 2B, and 3 isozymes were

transiently expressed in human HEK 293 cells. SERCA enzyme activity in microsomes isolated from transfected cells was measured by various assays, including γP32 -ATP hydrolysis, an enzyme-coupled spectrophotometric method, and an inorganic phosphate spectrophotometric assay. In each experiment, known SERCA inhibitors, thapsigargin (100nm) and 2,5-di-(*t*-butyl)-1,4-benzohydroquinone (10 μM), were included as positive controls. Results of these studies showed that 7,12-dimethylbenz-(a)anthracene (DMBA) partially inhibited the cloned SERCA 1, 2A, and 3 isozymes, but none of the other PAHs, including benoz(a)pyrene, 9,10-dimethylanthracene, dibenz(a,h)anthracene, dibenz(a,c) anthracene, benz(a)anthracene, benzo(e)pyrene, or anthracene caused significant inhibition at concentrations up to 10 μM. These results suggest that PAHs may inhibit SERCA enzymes in cells via indirect mechanisms. Supported by NIH RO1-ES05495

1655 URINARY EXCRETION KINETICS OF PYRENE AND BENZO(A)PYRENE METABOLITES IN RATS

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Knowledge of the excretion kinetics of a urinary metabolite is essential for its adequate use as a bioindicator of exposure. The detailed time profiles of 1-hydroxypyrene (1-OHP) and benzo(a)pyrene (BaP) metabolites excretion were studied in male Sprague-Dawley rats injected i.v. with 15 μmol pyrene/kg or 40 μmol BaP/kg. To examine the importance of biotransformation on these kinetics, other rats were exposed to 4 μmol 1-OHP/kg or 2 μmol/kg of a mixture of BaP metabolites. Urine samples were collected at frequent intervals over 48 or 96 hr. For all metabolites, similar profiles were obtained when injecting the parent compound or the metabolite. Excretion of 1-OHP was biphasic and a first order apparent half life ($t_{1/2}$) of 6.9 and 6.6 hr was calculated for the second phase when injecting pyrene and 1-OHP, respectively. Two steps of elimination were observed for 3-hydroxyBaP (3-OHBaP) after injection of BaP or the metabolites with respective $t_{1/2}$ of 7.6 and 6.7 hr. Elimination of BaP-trans-4,5-dihydrodiol (BaP-4,5-diol) was more rapid and appeared linear. A $t_{1/2}$ of 3.0 and 3.6 hr was calculated when respectively injecting BaP and BaP metabolites. Results suggest that 1) biotransformation is not a rate-limiting step in the excretion of 1-OHP, 3-OHBaP and BaP-4, 5-diol after injection of parent compounds and 2) similar $t_{1/2}$ obtained for 1-OHP and 3-OHBaP suggests that 1-OHP could be a good surrogate for 3-OHBaP. (Supported by the Canadian Network of Toxicology Centres)

1656 IN SITU AND IN VITRO INTESTINAL METABOLISM OF [G-^{3}H]-BENZOAPYRENE-9-GLUCURONIDE (BAP-9G) AND [G-^{3}H]-BAP-9-SULFATE (BAP-9S) IN CHANNEL CATFISH

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As part of a study of the trophic transfer of polycyclic aromatic hydrocarbons and their metabolites, we have examined the intestinal bioavailability and biotransformation of BaP-9G and BaP-9S. For *in situ* experiments, 10 and 40 μM concentrations of [G-^{3}H]-BaP-9G or [G-^{3}H]-BaP-9S were infused into an isolated perfused intestinal segment and the perfusion continued for 1 hour. Blood, mucosa and the remaining infusate were collected and analysed for [^{3}H] by counting and for chemical form by extraction and ion-pair reverse phase HPLC with radiochemical and fluorescence detection. In separate studies, the *in vitro* hydrolysis of 4-methylumbelliferylglucuronide (4MUG), BaP-9G, 4-MU-sulfate (4MUS) and BaP-9S were measured in intestinal mucosal microsomes at pH 4.5 (glucuronidase) and 7.0 (sulfatase), by measuring the fluorescence of the phenolic products at alkaline pH. *In situ*, both BaP-9G and BaP-9S were effectively taken up by mucosa and transferred into blood, in a dose-dependent manner. More than 99% of the extractable [^{3}H] in blood was intact BaP-9G or BaP-9S as administered, whereas in mucosa 3–13% (BaP-9S) or 0–8% (BaP-9G) of the extractable [^{3}H] was 9OHBaP, indicating slow hydrolysis. *In vitro* studies showed that compared with PAPS-sulfotransferase and UDP-glucuronosyltransferase, sulfatase and β-glucuronidase activities with 4MU or 9OHBaP conjugates were very low and K_ms were higher for hydrolysis than conjugation. The sulfatase K_m for BaP-9S was 11.2 ± 2.9 μM (mean ± S.D., n=5) and V_{max} was 2.5 ± 0.6 pmol/min/mg protein. Thus, conjugates of BaP-phenols can be absorbed intact into the blood from the diet. Supported in part by USPHS ES 05781.

1657 DIFFERENTIAL EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) AND BENZO(A)PYRENE (BAP) ON GENE EXPRESSION IN HUMAN PLACENTAL TROPHOBLAST CELLS

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Exposure to polychlorinated biphenyls or cigarette smoke during pregnancy has significant detrimental effects on fetoplacental development. BaP is the major polycyclic hydrocarbon in cigarette smoke, while TCDD is the reference compound for the toxicities of halogenated hydrocarbons. This study investigated whether TCDD and BaP disrupt the expression of important trophoblast growth regulatory genes in an established human trophoblastic choriocarcinoma cell line JEG-3. Northern blot analysis showed that cytochrome P450 1A1 mRNA expression was induced by both TCDD and BaP in a concentration- and time-dependent manner. Exposure of JEG-3 cells to BaP significantly increased the level of transforming growth factor (TGF)-β1 mRNA while the protooncogene c-myc mRNA level was decreased in a concentration- and time-dependent manner; TGF-α mRNA, however, was unchanged by BaP exposure. In contrast, TCDD treatment had no significant effect on the steady state mRNA levels of TGF-α, TGF-β1 and c-myc. In summary, BaP has a more disruptive effect than TCDD on the important growth regulatory genes TGF-β1 and c-myc in human placental cells. Moreover, data suggest that different mechanisms may be involved in TCDD and BaP toxic effects on human placenta. (Supported by NIEHS 07375)

1658 EFFECT OF NEONATAL EXPOSURE TO DIETHYLSTILBESTEROL (DES) ON BENZO(a)PYRENE (BaP)-DNA ADDUCT LEVELS IN RAT REPRODUCTIVE TISSUES

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This study examines the formation of B(a)p-DNA adducts and their role in carcinogenesis in the male reproductive system of rats. Offspring of Sprague-Dawley rats were neonatally treated on days 2, 4, and 6 postpartum with 1.45 *u*moles of DES. Ten weeks after birth, the adult rats were challenged with ^{3}H BaP (10 *u*mol/kg), and sacrificed 2 hours after exposure. Prostate, testes and liver tissues were removed and DNA was isolated. The DNA samples were digested with Spleen phospho-diesterase, Micrococcal nuclease and subjected to ^{32}P-postlabeling. The labeled adducts were separated by TLC and quantified by scintillation counting. Some adducts were separated by reverse phase HPLC also. The adduct levels in DES animals appeared to be less than those in controls for prostate and testes compared to liver samples. Earlier studies showed relatively lower levels of 7,8-epoxide GSHs in these tissues, compared to other conjugated metabolites. These findings indicate that the availability of reactive metabolites will influence the formation, persistence and concentrations of adducts. Furthermore, reduced levels of adducts suggest a possible chemopreventive effect from DES.

1659 ACUTE AND SUBCHRONIC TOXICITY OF BENZO(A)PYRENE IN F-344 RATS

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We have studied the acute and subchronic oral toxicity of benzo(a)pyrene in male and female F-344 rats. Acute doses of 0, 100, 300, 600, and 1000 mg/kg were administered in peanut nut oil by gavage. Subchronic doses of 0, 5, 50 and 100 mg/kg were administered in the animal diet over a 90 day period. After the respective dosing periods, animals were sacrificed, trunk blood collected and selected organs were removed for histopathological examination. None of the seven blood elements examined (WBC,RBC,Hgb, Hct%,etc.) showed any significant changes from the control for the acute or subchronic studies. Blood enzymes (AST,ALT,BUN) were not significantly altered in the acute studies. Acute doses of BaP produced no effect on selected tissues examined (liver, kidney, testes, prostate, ovaries, stomach), however the mid and high doses of BaP in the 90 day study showed significant kidney abnormalities (tubular casts) in males only. These studies indicate that the acute toxicity of BaP may be relatively low, but subchronic exposures to BaP at relatively low doses may induce non-carcinogenic kidney abnormalities.

1660 BIOAVAILABILITY OF CHEMICAL COMPONENTS OF SOIL CONTAMINATED WITH MANUFACTURED GAS PLANT RESIDUE

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The urinary excretion of pyrene metabolites and chemical:DNA adduct formation in lung and forestomach tissue was investigated in mice following the ingestion of soil contaminated with manufactured gas plant residue (MGP). Contaminated soil was fractionated into particle sizes ranging from 1.0 mm to less than 150 μm. The polycyclic aromatic hydrocarbon (PAH) content of each soil sample was determined. Soil fractions were incorporated into powder diets (20 g soil/ 100 g diet) and fed to groups of female CD1 mice for a total of 15 days. Animal body weight and food consumption was monitored throughout the study. Urine was collected on day 14 of diet administration and the level of pyrene metabolites excreted was determined using HPLC equipped with fluorescence detection. DNA isolated from lung and forestomach tissues was analyzed for chemical:DNA adducts using ^{32}P-postlabeling and multidimensional TLC. Pyrene metabolites were detected in urine of mice ingesting diets containing soil. The amount of the ingested pyrene excreted in urine ranged from 0.4-4.0%. Mice ingesting particles less than 150μm in size had the highest level of pyrene in urine. Chemical:DNA adducts were detected in both lung and forestomach tissues of animals ingesting diets containing contaminated soil. Similar to pyrene excretion, the highest level of adduct formation was associated with particles less than 150 μm. Adduct levels were approximately 5 times higher than those induced with larger particle sizes. These results suggest that particles less than 150 μm play an important role in the genotoxic potentcy of soils contaminated with MGP residue. (Supported by EPRI RP-2963).

1661 DEPLETION OF GLUTATHIONE BY POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN HUMAN PERIPHERAL BLOOD T CELLS

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Previous studies in this laboratory have shown that PAHs alter Ca^{2+} homeostasis and inhibit activation of both B and T lymphocytes obtained from rodents and humans. Other investigators have demonstrated that intracellular Ca^{2+} may increase in lymphocytes in response to oxidizing conditions, such as ionizing radiation (Schieven *et al, J. Biol. Chem.* **268**, 16688, 1993). In addition, oxidants produced by cells (such as hydroxyl radical, H_2O_2, or superoxide anion) as well as oxidizing chemicals have been shown to increase intracellular Ca^{2+} and decrease intracellular levels of glutathione (GSH). Studies in our laboratory have shown that a 9 hr incubation with PAHs such as 7,12-dimethylbenz(a)anthracene (DMBA) and benzo(a)pyrene (BaP) decrease intracellular GSH in the A20.1 murine B lymphoma cell line (Yuan *et al, Fund. Appl. Toxicol.* **23**, 336, 1994). Because PAHs alter activation and intracellular signaling in T cells, we measured GSH levels in 6 day phytohemagglutinin-stimulated T cell blasts exposed to PAHs for various times. GSH was measured using both the Tietze and Reed assays. We demonstrated in T cell blasts that 1–10 μM BaP, DMBA, benzo(e)pyrene, and anthracene disrupt intracellular glutathione homeostasis. BaP, DMBA, and perhaps BeP were found to deplete GSH after both 6 hr and 18 hr incubations. These data suggest that oxidative mechanisms may play a role in PAH-induced immunotoxicity in human T cells. Supported by NIH RO1 ES-05495.

1662 THE ARYL HYDROCARBON RECEPTOR IS A SERUM-INDUCIBLE GENE IN MURINE 3T3 FIBROBLASTS

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We have investigated the expression of the aryl hydrocarbon receptor (AhR) in Swiss 3T3 fibroblasts. The proliferative state of these cells is regulated by serum growth factors present in the culture medium. Fibroblasts growing exponentially in 10% serum expressed high levels of AhR protein, as determined by immunoblot analysis of cell lysates. Growth arrest, resulting from serum-starvation, resulted in a marked down-regulation of AhR protein. Serum stimulation of growth arrested fibroblasts resulted in induction of the AhR after a lag period of 18–22 hours, concomitant with entry into the G_2 phase of cell cycle. To elucidate mechanisms of serum regulated expression of the AhR, an AhR promoter/reporter gene construct was transiently transfected into 3T3 cells. The AhR promoter drove strong expression of reporter gene activity in serum stimulated cells, but not in growth-arrested fibroblasts. These data show that expression of the AhR is transcriptionally regulated in a cell cycle-dependent manner. Supported by RO1 ESO6086.

1663 MODULATION OF GROWTH-RELATED GENE EXPRESSION IN PRIMARY CULTURES OF RAT HEPATOCYTES BY BENZO(A)PYRENE AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

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Benzo(a)pyrene (BaP) and 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) exert prominent biochemical and toxic effects in multiple organs, including liver. The actions of these chemicals may involve deregulation of growth-related gene expression. To test this hypothesis, hepatocytes were isolated from adult female Sprague-Dawley rats by a two-step collagenase perfusion technique and seeded on collagen coated petri dishes. Hepatocytes were allowed to attach for 1 hr prior to chemical exposure and the patterns of chemically-induced c-Ha-ras, c-myc, c-fos and c-jun expression monitored by Northern analysis. Challenge of hepatocytes with 3 μM BaP or 1 nM TCDD for 4 hr increased c-Ha-ras and c-myc, but decreased c-fos and c-jun mRNA levels. A 1 hr pretreatment of cells with 10 nM α-naphthoflavone, an aryl hydrocarbon receptor antagonist and inhibitor of cytochrome P450, antagonized the effects of BaP and TCDD on gene expression. Collectively, these data implicate differential regulation of growth-related genes in response to aromatic hydrocarbons and suggest that the overall protooncogene response profile most likely involves aryl hydrocarbon receptor-dependent mechanisms. (Supported by NIH grants ESO4917 and ES 00213).

1664 POLYCYCLIC AROMATIC HYDROCARBON CONTRIBUTION TO COAL TAR-INDUCED HEPATOCARCINOGENICITY

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Manufactured gas plant residues (MGPR), coal tars are complex mixtures of polycyclic aromatic hydrocarbons (PAHs) and include compounds such as benzo[a]pyrene which induce P450-dependent activities responsible for metabolic activation of PAHs to form DNA adducts which are associated with initiation of carcinogenesis. By GC/MS analysis, PAHs constituted only 18% by weight of the MGPR used in this study. The crude MGPR and a reconstituted mixture of PAHs resembling MGPR-PAH content were administered by i.p. injection to 15 day-old B6C3F1 male mice. Ethoxyresorufin *O*-deethylase and methoxyresorufin *O*-deethylase activities for the high-dose crude MGPR mixture (1071 mg/kg) were 4907 pmol/min/mg and 1378 pmol/min/mg, respectively and, for the reconstituted mixture (193 mg/kg), 1740 pmol/min/mg and 595 pmol/min/mg, respectively. Using the [^{32}P]-postlabeling assay, the relative adduct level (RAL = adducted nucleotides per 10^7 unadducted nucleotides) for the crude MGPR was 4.3, whereas the RAL for the corresponding reconstituted mixture was 0.47. After exposure to the crude MGPR for 26, 39 and 52 weeks, the percentage of mice with hepatic tumors were 3%, 45% and 43%, respectively. The reconstituted PAH mixture did not significantly increase tumor incidence at any time point, indicating that these PAHs alone are not responsible for the carcinogenicity of the crude MGPR.

1665 INDUCTION OF HEPATIC CYP1A2 BY ACENAPHTHYLENE IN MICE: AGE-DEPENDENT EFFECTS

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Previous studies have shown that acenaphthylene induced hepatic CYP1A2 gene expression in female B6C3F1 mice. Preliminary studies with several genetically-inbred strains of mice showed that the activity of acenaphthylene (300 mg/kg) as an inducer of CYP1A2-dependent methoxyresorufin *O*-demethylase (MROD) was highly variable. The data suggested possible age-dependent effects and therefore 7, 14, 21, 28 and 42 day-old male B6C3F1 mice were treated with a single dose of acenaphthylene (300 mg/kg) and hepatic microsomal MROD activity was determined. The results showed that

MROD activity in corn oil-treated (control), 7 and 14 day-old mice was 63 ± 11 and 50 ± 13 pmol/min/mg and after treatment with acenaphthylene there was approximately at 10-fold increase MROD activity. In contrast, less than a 3-fold induction response was observed 21, 28 and 42 day-old mice. The loss of inducibility was primarily due to the age-dependent increase of basal MROD activity from ≤ 63 pmol/min/mg (7 and 14 day-old mice) to 167 to 282 pmol/min/mg for the 21 to 42 day-old animals. Current studies are focused on further characterizing the age-dependent effects of acenaphthylene and related compounds on hepatic CYP1A2 gene expression in genetically inbred male and female mice and other rodent species.

1666 RELATIVE INDUCTION POTENCY OF SELECTED POLYNUCLEAR AROMATIC HYDROCARBONS (PAHS) IN ENVIRONMENTAL SAMPLES USING THE H4IIE CELL BIOASSAY

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Induction of ethoxyresorufin O-deethylase (EROD) activity in H4IIE cells is a useful assay to assess the toxic equivalents (TEQs) of complex mixtures in environmental samples. Benzo(a)pyrene (BAP), dibenz(a,h)anthracene (DBA), benzanthracene (BA), benzo(k)fluoranthrene (BkF), and benzo(b)-fluoranthrene (BbF) are carcinogenic polynuclear aromatic hydrocarbons (PAHs) found in environmental samples and the induction potency of these PAHs relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was determined in H4IIE cells. Using probit analysis, the induction-TEFs were 0.000843, 0.00359, 0.000853, 0.0256, 0.00722 for BAP, DBA, BA, BkF, and BbF, respectively. Reconstituted PAH mixtures (744 to 4466 ng/g total PAHs) with constant percentages of BAP (4.5%), DBA (3.5%), BA (2.4%), BkF (3.7%), and BbF (3.5%) yielded bioassay-derived induction-TEQs which ranged from 0.52 to 1.44 ng/g. Oysters exposed to the same PAH mixture for 30 days differentially accumulated the PAHs with time and bioassay-derived induction-TEQs of oyster extracts ranged from 0.99 to 6.14 ng/g. In contrast, the calculated induction-TEQs were 0.072 to 10.0 ng/g, suggesting that the concordance between the calculated and observed induction-TEQs was dependent on the sample. This indicated the presence of other PAH inducers in some of the samples, and these are currently being investigated.

1667 TRANSCRIPTION-SPECIFIC REPAIR OF BPDE ADDUCTS IN THE tk GENE OF THE MMTV CONSTRUCT

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Mouse L1.4-3 cells, which contain a stably integrated construct consisting of the herpes simplex virus thymidine kinase gene (*tk*) linked to mouse mammary tumor virus long terminal repeats (MMTV-LTR), were dosed with benzo[a]pyrene diol epoxide. The *tk* gene in these cells is constitutively expressed at low levels, but transcription is increased >50 fold upon induction with dexamethasone. BPDE was shown to inhibit *tk* mRNA in a dose dependent manner suggesting single-hit kinetics, as previously shown for UV photoproducts. When the cells are allowed to repair in the absence of dexamethasone, there is very little transcription after 24 hours of repair. However, when the cells are allowed to repair in the presence of dexamethasone, the *tk* transcription recovers to >60% of undamaged levels within 24 hours (similar to bulk repair). These results suggest that the blockage of transcription by BPDE adducts is preferentially removed when the *tk* gene is in a transcriptionally active state. Currently we are conducting quantitative PCR with Mouse L1.4-3 genomic DNA to determine specific regions of the MMTV construct that are damaged by BPDE. These specific sites will be further characterized with respect to chromatin structure. (Supported by US DOE Contract DE-AC06-76RLO 1830)

1668 CONTROLLED RECRUITMENT OF MONOCYTES/ MACROPHAGES TO THE CENTRAL NERVOUS SYSTEM (CNS) VIA TRANSGENIC EXPRESSION OF MCP-1

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Monocyte chemoattractant protein-1 (MCP-1), a member of the "C-C" family of chemokines, induces chemotaxsis of monocytes at subnanomolar concentrations *in vitro*. We expressed MCP-1 constitutively under the control of the myelin basic protein (MBP) promoter in transgenic mice. Paralleling the temporal increase in mRNA levels, we observed a mononuclear cell infiltrate by light microscopy in discrete areas of the CNS of 3 wk-old transgenic mice. The recruited cells were predominantly in a perivascular orientation in meninges, choroid plexus, and white matter. Abundant MCP-1 mRNA was localized throughout the white matter by isotopic *in situ* hybridization. Most of the cellular infiltrates in the brains of the transgenic mice stained positively with the macrophage markers, Mac-1 and F4/80, but were largely negative for T and B cell markers. Luxol-fast blue staining of myelin resembled controls, indicating that transgene expression with monocyte recruitment did not affect myelinogenesis. The infiltrate, present in Virchow-Robin spaces with extension into adjacent neuropil, had ultrastructural features of quiescent macrophages. Small numbers of apoptotic cells and a rare lymphocyte were associated with the inflammatory infiltrate. The infiltrate was significantly amplified following endotoxin, suggesting that the recruitment properties of MCP-1 can be potentiated by additional factors. The results of this study indicate that MCP-1 protein is capable of activating monocyte and macro-phage recruitment *in vivo*.

1669 MECHANISM OF TOXICITY OF 5-FLUOROURACIL IN COLON TUMOR CELLS

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5-Fluorouracil (FUra) is a prominent antineoplastic agent used broadly in the treatment of colorectal cancer. Its metabolic conversion to 5-fluorouridine 5′ of-triphosphate with subsequent incorporation into RNA and/or the formation of 5-fluorodeoxyuridylic acid, a potent inhibitor of thymidylate synthase, are thought to underlie its mechanism of action. Important in tumor development is the presence of cytokines that regulate the production of mediators controlling tumor growth, invasion and metastasis. One critical cytokine produced by lymphocytes infiltrating tumors is γ-interferon. We have found that γ-interferon (100 U/ml) stimulates DLD-1 human colon carcinoma cells to produce nitric oxide, an important vasoactive mediator. Nitric oxide is produced from the NADPH-dependent oxidation of l-arginine by the enzyme nitric oxide synthase. We have found that γ-interferon stimulates expression of the type II isoform of this enzyme in DLD-1 cells. FUra, at concentrations that were not cytotoxic (0.5–5 μM), was found to suppress γ-interferon induced nitric oxide production by the cells. Time course studies revealed that treatments with FUra for as little as 3 hrs was sufficient to inhibit nitric oxide production. The ability of FUra to interfere with the action of γ-interferon on colon tumor cells may be an important mechanism of action of this chemotherapeutic agent. (NIH ES 05022).

1670 CELL CYCLE-BLOCKING AGENTS REDUCE NECROTIC CELL DEATH CAUSED BY ALKYLATING HEPATOTOXINS

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Liver arylation by acetaminophen (APAP) and alkylation by N-nitrosodimethylamine (DMN) produce DNA damage followed by necrotic cell death. Our working hypothesis states that when hepatocytes enter cell cycle prior to undergoing compensatory proliferation, those cells with substantial unrepaired DNA damage die by necrosis. This hypothesis predicts that agents which block the cell cycle in G_2/M (vincristine) or G_1 (mimosine) will attenuate hepatotoxicity of APAP and DMN. APAP was examined in male NIH Swiss mice and DMN in male ICR mice weighing 25–35 g. Mice were treated with vehicle (control), hepatotoxin ip (APAP 600 mg/kg in saline or DMN 20 mg/kg in saline), hepatotoxin and a nontoxic dose of vincristine (1 mg/kg in saline ip) or mimosine (100 mg/kg in saline ip) 2 hr later, or vincristine or mimosine alone. Serum and liver were collected at 24 hr for measurement of alanine aminotransferase (ALT) activity and DNA damage. Vincristine treatment significantly decreased the APAP-induced mean ALT elevation (3654 ± 1859 vs 8073 ± 6774 U/l, $p < 0.05$) and mimosine treatment significantly decreased the DMN-induced mean ALT elevation (1571 ± 886 vs 3771 ± 5556 U/l, $p < 0.05$). Results support the hypothesis that DNA damage plays a casual role in necrotic cell death produced by arylating and alkylating hepatotoxins (NIH ES 06345).

1671 ALTERATIONS IN MUSCARINIC RECEPTOR mRNA LEVELS IN RAT BRAIN FOLLOWING REPEATED ORGANOPHOSPHATE EXPOSURE

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Repeated exposure to organophosphate pesticides (OPs) causes a decrease in muscarinic acetylcholine receptors (MRs) in the brain, with resultant negative effects on cognitive function, especially memory and learning. There are five MR subtypes (m1-m5), and each subtype may be differentially affected by OPs. This study was designed to test the effects of OP exposure on the expression of m1, m2, and m3 mRNAs in brain, before and after a 28 d recovery period. Adult male Sprague-Dawley rats were orally administered the OP disulfoton (2mg/kg/day) for 14 d, and tissues were analyzed for expression of MR mRNAs by RNA-RNA solution hybridization. The treatment caused a 28% and 81% decrease in cortical 3H-QNB binding and acetylcholinesterase activity, respectively; after recovery, these levels had returned to 99% and 92%, respectively, relative to controls. Disulfoton treatment caused a significant decrease in m1 mRNA levels in hippocampus (23%), but did not change m1 levels in the cortex, striatum, medulla, or cerebellum. m2 mRNA was significantly decreased in both hippocampus (24%) and medulla (19%), but not in cortex, striatum, or cerebellum. m3 mRNA levels were significantly decreased in cortex (10%), but no change was observed in hippocampus, medulla, or cerebellum. After recovery, no differences in m1 or m3 mRNA levels were observed in any tissue examined, whereas the decrease in m2 mRNA in the hippocampus remained significant (29%). These results indicate that OP exposure can differentially regulate mRNA levels for MR subtypes in different brain areas, and suggests that co-ordinate regulation of MR receptors and mRNA (m1 and m2) is strongest in the hippocampus. Furthermore, the loss of hippocampal m2 mRNA appears to be long lasting. (Supported in part by ES-04696 and NIEH ES-07032).

1672 MODIFICATION OF THE DIFFERENTIAL DISPLAY TECHNIQUE: USE IN THE IDENTIFICATION OF TCDD-REGULATED GENES

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Differential mRNA display is a powerful PCR-based technique that can be applied to the study of gene expression and regulation. Typically, the differential display technique has employed the incorporation of ^{35}S, ^{32}P, or ^{33}P to label newly synthesized cDNA products. These products are then displayed on a sequencing gel and analyzed by auto-radiography. Modifications on this technique have been made in our laboratory in the search for TCDD-responsive genes. Firstly, the use of radioisotopes for labeling and visualization of cDNA products has been eliminated. Instead, a fluorescent dye is used to stain the gel after electrophoresis. cDNA products can then be immediately visualized under UV light and analyzed by Fluorimager. This modification eliminates the exposure hazard associated with the use of radioisotopes. The second modification involves the replacement of the large sequencing gels with a small, nondenaturing 4–15% gradient polyacrylamide gel. This gel is more manageable and allows for the visualization of cDNA products ranging from 100 bp to 2.0+ kb, compared to sequencing gels that only allow the resolution of products under 600 bp. Finally, problems associated with PCR reamplification of rehydrated gel slices are eliminated since the smaller wet gel can be used directly to visualize cDNA products. In summary, these modifications of the differential display technique allow for more rapid results and greater manageability. (Supported by NIH Grant ES02515, Center Grant ES01247, & Training Grant ES07026.)

1673 CLONING OF A SECOND MAJOR UDP-GLUCURONOSYL TRANSFERASE (UGT) mRNA INDUCED BY OLTIPRAZ IN FEMALE F344 RAT LIVER: IDENTIFICATION AS UGT1A2

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Previously we have found that the steady state UGT1A1 mRNA is increased > 50-fold in livers of female F344 rats treated with oltipraz, a chemoprotective agent thought to act through induction of chemical-detoxifying enzymes. Studies with two different SV40 tsA-immortalized adult rat liver clonal cell lines, LCS-3 and LG-2, showed that UGT1A1 message is elevated in LG-2 but not LCS-3 cells 24 hrs after addition of oltipraz (100 μM). Analysis of mRNA from oltipraz-treated LCS-3 cells for UGT1 family transcripts using a "pan-UGT1" probe corresponding to conserved exons 2–5 revealed the presence of a second responding UGT1 mRNA. To identify this RNA, a lambda ZAP cDNA library was constructed using RNA from LCS-3 cells treated for 24 hours with oltipraz (100 μM), and the library was screened with the pan-UGT1 probe. UGT1 transcripts were found to be highly abundant with approximately 75 positives per 50,000 clones. Fifteen clones designated LC-1 to LC-15 were analyzed by Southern blotting; none were found to represent UGT1A1 indicating low UGT1A1 expression. Specific probes for 9 clones with the longest inserts were prepared and used for Northern analyses of RNA from untreated and oltipraz-treated LCS-3 cells. Each of the 9 showed high oltipraz inducibility. A corresponding analysis of liver RNA from control and oltipraz-treated F344 rats revealed > 40 fold inducibility *in vivo*. Terminal nucleotide sequence analysis of several clones with the longest inserts identified the unknown UGT1 cDNA as UGT1A2. This is the first report of the cloning of the rat UGT1A2 mRNA. Its role in chemoprotective mechanisms remains to be determined. Supported by the Thomas F. and Kate Miller Jeffress Trust.

1674 CHARACTERIZATION OF THE ANATOMIC LOCALIZATION OF CYCLOOXYGENASE (COX)-1 AND COX-2 IN THE RAT BRAIN WITH AND WITHOUT AN IMFLAMMATORY STIMULUS

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Prostaglandins are thought to play a role in many nervous system functions such as sleep regulation, maintenance of basal body temperature and the transmission of pain. The principal enzyme in prostaglandin biosynthesis is cyclooxygenase (COX) which exists in either the constitutive (COX-1) or inducible (COX-2) form. Both isoforms have been shown to be expressed in the rat brain. The objective of the present work was to characterize COX-2 expression and to determine its interrelationship with COX-1 in both normal healthy and diseased brain models. Rats (4/sex/time point/group) were given IV injections of either saline or lipopolysaccharide (LPS; 2.5 mg/kg), anesthetized at 1, 3 or 6 hrs and then perfused (lactated Ringers and 10% formalin). Brains were examined for COX-1 and COX-2 antigen labelling and mRNA expression by immunohisto-chemical and in situ hybridization techniques. Peripheral blood was collected prior to sacrifice for analysis of COX-1 and COX-2 expression as a correlate to the LPS-induced inflammatory response. The results of this study will be presented.

1675 2-BROMO-6-(GLUTATHION-S-YL)HYDROQUINONE-MEDIATED CYTOTOXICITY IN RENAL PROXIMAL TUBULAR EPITHELIAL CELLS: A POSSIBLE ROLE FOR CALCIUM

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We have previously shown that 2-Br-6-(glutathion-*S*-yl)-hydroquinone [2-Br-6-(GSyl)HQ] causes a transient, time- and dose-dependent decrease in intracellular pH (pH_i) which reaches a nadir about 45 min. after exposure of cells to 200 μM 2-Br-6-(GSyl)HQ, ($\Delta pH \sim 0.5$–0.6 units). pH_i is intimately related to intracellular calcium $[Ca^{2+}]_i$ and may act as an independent regulator of $[Ca^{2+}]_i$. In addition, both reactive oxygen species and calcium (Ca^{2+}) are involved in toxic and ischemic renal tubular cell injury. In the present study we investigated the role of Ca^{2+} in the nephrotoxicity of 2-Br-6-(GSyl)HQ in renal proximal tubular epithelial cells (LLC-PK_1). Buffering $[Ca^{2+}]_i$ with 1,2-*bis*-(2-aminophenoxy) ethane-*N,N,N',N'*-tetraacetic acid (50 μM) in cells treated with 200 μM 2-Br-6-(GSyl)HQ provided significant protection against cell injury, as evidenced by enhanced neutral red uptake compared to controls. This protective effect was observed at time points beyond 30 min, and continued for at least 10 hours after treatment. The use of 8-(*N,N*-dimethylamino)octyl-3,4,5-trimethoxybenzoate hydrochloride (0.1 mM), which prevents the mobilization of Ca^{2+} from intracellular storage sites, had little effect on cytotoxicity. However, exclusion of Ca^{2+} from the medium or the addition of verapamil, a voltage-gated Ca^{2+} channel blocker, also failed to enhance cell viability. Taken together, the data suggest a possible role for a disruption in cellular Ca^{2+} homeostasis in the cytotoxicity of 2-Br-6-(GSyl)-HQ, the mechanism of which requires further study.
(Supported by ES 07359).

1676 *IN VITRO* ANALYSIS OF LIGAND DEPENDENT CHANGES IN Ah-RECEPTOR-DRE INTERACTIONS

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6-Methyl-1,3,8-trichlorodibenzofuran (MCDF) and related alkyl polychlorodibenzofurans (PCDFs) are relatively non-toxic analogs of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) which exhibit comparable *in vitro* and *in vivo* antiestrogenic and antitumorigenic activities. However, MCDF is a weak aryl hydrocarbon receptor (AhR) agonist for the traditional toxic and biochemical responses induced by this class of chemicals. In previous studies, treatment of Ah-responsive MCF-7 human breast cancer cells with 100 nM MCDF inhibited 17β-estradiol (E2)-induced proliferation and the secretion of the 34-, 52- and 160-kDa proteins. In addition, MCDF antagonized TCDD-mediated immunotoxicity and induction of CYP1A1 gene expression. Although MCDF did not induce CYP1A1 mRNA in the MCF-7 or Hepa 1c1c7 cells lines, incubation of nuclear extracts from MCF-7 cells treated with MCDF with a [^{32}P]-dioxin responsive element (DRE) oligonucleotide gave a retarded band in a gel retardation assay. To further analyze this interaction, the ligation-mediated polymerase chain reaction has been utilized to investigate the interactive differences between TCDD and MCDF on the Hepa 1c1c7 CYP1A1 5'-regulatory region by determining methylation and nucleosome DNA footprints. Preliminary results demonstrate that the guanine on the sense strand of the DRE (CGCACT), which is protected in TCDD-treated cells, is not protected in cells treated with MCDF. These data suggest that the failure of MCDF to induce CYP1A1 gene expression may be due to the differences in the *in vivo* binding of the AhR to the DRE.

1677 REGULATION OF COLLAGEN BIOSYNTHESIS AND PROCOLLAGEN TRANSCRIPTION IN A RAT LUNG MODEL OF PULMONARY FIBROSIS

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The inflammatory response leading to collagen deposition and subsequent pulmonary fibrosis is often considered analogous to the process of wound healing. Early in the typical skin wound healing process, there is a transient increase in Type III collagen synthesis and deposition followed by a progressive increase in Type I collagen deposition, which continues until resolution of the inflammatory response and subsequent scar formation or normal healing. We hypothesized that a similar sequence of events occurs during experimentally induced pulmonary fibrosis. To test this hypothesis we administered bleomycin intratracheally to rats as a fibrogenic stimulus, and studied the time course of the response. We quantified apparent collagen synthesis rates using lung slices, the ratio of type I:III collagen synthesized by the lung slices, and the whole lung content of mRNAs for procollagens I and III. We found elevated collagen synthesis rates as early as 3–4 days after administration of bleomycin. Collagen type ratios remained constant for about 5–6 days at the control level of 2:1, then increased to as high as 5:1 at day 21. Procollagen mRNA contents, expressed as a ratio of type I:III mRNAs, were consistent with the protein synthesis data and the observed ratio of collagen types being made by the lungs at the various time points evaluated. We conclude that our original hypothesis, that there would be a transient increase in type III collagen synthesis and deposition preceding an increase in type I collagen in the early stages of lung fibrosis, is not demonstrable when the response of the whole lung is examined.

1678 ACTIVATION OF THE TRANSCRIPTION FACTOR NF-κB BY *S*-(1,2-DICHLOROVINYL)-L-CYSTEINE (DCVC) IN LLC-PK1 CELLS

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Activation and nuclear translocation of NF-κB are mediated by reactive oxygen intermediates (ROI). NF-κB resides in the cytosol associated with its inhibitory protein IκB in the resting state and once activated, IκB dissociates and NF-κB migrates to the nucleus (transactivation). In the nucleus, NF-κB induces stress-responsive genes. The toxic effects of the nephrotoxin *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC) are inhibited by antioxidants, indicating possible involvement of ROI in its mechanism of action. We investigated the activation of NF-κB by DCVC in LLC-PK1 cells, a renal epithelial cell line. Gel-mobility-shift assays indicated activation of NF-κB in nuclear extracts of cells incubated with DCVC. Specificity control experiments showed competition between binding of NF-κB to its consensus, radiolabeled oligonucleotide by excess unlabeled oligonucleotide, but not by an excess of a mutated oligonucleotide. Increased activation of NF-κB was observed with increasing concentrations of DCVC. Activation of NF-κB was rapid: activation was observed within 15 min after treatment of cells, and peak activation occurred 1–2 h after treatment of cells. DCVC undergoes β-lyase-dependent biotransformation, and the β-lyase inhibitor (aminooxy)acetic acid (AOA) blocked DCVC-induced toxicity. AOA partially inhibited activation of NF-κB by DCVC. Activation was also inhibited by the antioxidants *N*-acetyl-L-cysteine and pyrrolidinedithiocarbamate. Transactivation of NF-κB by DCVC was observed in cells transfected with a plasmid containing NF-κB binding sites linked to a luciferase reporter gene. Luciferase activity was partially inhibited in transfected cells treated with DCVC and AOA. Activation of NF-κB appears to be an early event in the cascade of events leading to DCVC-induced toxicity. (Supported by NIEHS grant ES03127)

1679 PCR-CLONING OF CYTOCHROME P-450IA cDNAs FROM THE LIVERS OF TILAPIA AND COMMON CARP

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CYPIA genes are inducible by some halogenated aromatic hydrocarbons (HAHs) and polyaromatic hydrocarbon (PAHs), hence CYPIA induction has become a useful biomarker of exposure to these organic environmental contaminants. To study CYPIA gene expression in fish, we used reverse transcription-polymerase chain reaction (RT-PCR) to clone the cytochrome P450IA (CYPIA) cDNAs of common carp (*Cyprinus carpio*) and tilapia (*Tilapia mossambicus*). Two pairs of degenerate primers were designed from the conserved regions of the teleost CYPIA cDNA sequences retrieved from the Genebank. Using these primers for RT-PCR, the partial CYPIA cDNAs of common carp (0.6 kb) and tilapia (0.4 kb) were amplified from uninduced liver. Two CYPIA cDNA sequences were identified in common carp whereas one CYPIA cDNA sequence was identified in tilapia. To obtain the full length sequences of these CYPIA cDNAs, the rapid amplification of the cDNA ends (RACE) approach has been adopted by using primers designed from the partial CYPIA cDNA sequences. Southern blot analysis and PCR results showed that there are two genes encoding for CYPIA in the genome of common carp.

1680 ALTERATION OF PROINFLAMMATORY GENE EXPRESSION AND HEPATIC MACROPHAGE POPULATIONS FOLLOWING DIMETHYLNITROSAMINE EXPOSURE *IN VIVO*

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Acute exposure to the hepatotoxicant dimethylnitrosamine (DMN) initiates a rapid inflammatory response characterized by hepatic IL-6 and metallothionein gene expression. We now demonstrate that chronic DMN exposure leads to a progression of immunocyte influx into the liver associated with tissue damage. Because we previously determined that tumor necrosis factor (TNF-α) is expressed following DMN exposure *in vivo*, we further hypothesized that TNF-α may indirectly exacerbate hepatotoxicity by mediating the accumulation and activation of immunocytes within inflammatory foci. We utilized TNF receptor knockout mice lacking either the p55, the p75, or both receptors to study the role of DMN-induced TNF-α signals in hepatic tissue damage and the formation of inflammatory foci. Mice were administered DMN (5 mg/kg) or PBS as vehicle daily for up to 2 weeks. Livers were excised and prepared for histopathologic evaluation of damage and for immunohistochemical determination of non-parenchymal cells within foci. In addition, we utilized novel macrophage population-specific probes identified from a subtraction library containing cDNAs expressed by inflammatory, GM-CSF-derived macrophages, but not quiescent, CSF-1-derived macrophages to characterize hepatic macrophages. Northern blot analysis of total liver RNA prepared from DMN exposed mice were hybridized against cDNA probes. Results demonstrated alterations in the molecular phenotype of hepatic macrophages following chronic DMN exposure *in vivo*. This work was supported by NIH Grant ESO-4348.

1681 SULFUR MUSTARD (HD) STRESS GENE INDUCTION IN TRANSFORMED HEP G2 CELLS

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HD cell stress gene induction profiles were investigated using the CAT Tox(L) Hep G2 assay system produced by Xenometrix, Boulder, CO. The HD dose range tested was 12.5, 25, 50, 100, and 200 μM for 1h and CAT gene induction was determined 24h after initial exposure. HD induced the xenobiotic response element (XRE), metallothionein II_A (MTII), c-*fos*, nuclear factor kappa binding response element (NFkBRE), heat shock protein 70kDa (HSP), cyclic AMP response element (cAMPRE), protein 53kDa response element (p53RE), growth arrested and DNA damage protein 153kDa (GADD153), growth arrested and DNA damage protein 45kDa (GADD45), and glucose regulated protein 78kDa (GRP78) greater than 2-fold. HD concentrations for onset of induction and peak induction of the specific genes were 200/200μM for XRE, 100/200μM for MTII, 25/200μM for c-*fos*, 25/50μM for NFkBRE, 100/200μM for cAMPRE, 25/100μM for p53RE, 100/200μM for GADD153, 50/200μM for GADD45, and 200/200μM for GRP78. Induction by HD reached the largest values (n = 5) with MTII, c-*fos*, NFkBRE, p53RE, and GADD153 at 13 ± 2, 15 ± 3, 7 ± 1, 28 ± 7 and 12 ± 1-fold (x ± S.E.), respectively. A time course of gene induction was studied at 2, 4, 8, and 16h. The earliest gene induced was metallothionein after 8h at the 100 μM dose. All stress genes found induced at the 24h post-induction time point were also induced at 16h. Di(cholorethyl) amine (HN2) had similar gene induction profiles as HD at the same concentration range.

1682 CYTOSKELETAL RECOVERY FOLLOWING TOXICANT-INDUCED NETWORK REDISTRIBUTION

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Exposure to 2,5-hexanedione (HD) causes a profound disruption of intermediate filaments (IF) in fibroblasts either by its direct action on IF or by secondary effects following alterations to other cytoskeletal proteins. Using immunofluorescent techniques we studied changes in the cytoskeleton during treatment with and recovery from HD, colcemid (CL), and cytochalasin D (CD), compounds which affect IF, microtubule (Mt) or microfilament (Mf) networks, respectively. In CD-treated cells there was a complete loss of Mf in less than 16 hours, dramatic changes in cell shape, and redistribution of the IF network within the new cell dimensions. Recovery of both the Mf and IF networks was complete within 24 hours after removal of CD. Treatment with CL altered Mt dynamics causing redistribution of IF but not Mf. There was also a change in the shape and size of CL-treated cells and the IF network was rearranged within the smaller cells, forming aggregates. The IF network had recovered within 24 hours after removal of the compound. During HD exposure there was loss of vimentin from the cell margins and formation of peri-nuclear aggregates. Mt and Mf were not affected. In HD-treated cells the IF network had not completely recovered even 120 hours after removal of HD. The profound disruption of IF distribution, long recovery time observed, and the differences between HD, CD, and CL exposure indicate that HD-induced alterations are not due to secondary changes in Mt or MF protein networks, but are due to the direct action of HD on the IF network.

1683 CHARACTERIZATION OF THE PROMOTER AND 5'-FLANKING REGION OF THE MURINE Ah RECEPTOR GENE

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The Ah receptor (AhR) is a ligand-dependent transcription factor which not only modulates gene expression but appears to be involved in mediating many of the toxic and biological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin and related chemicals. Although many studies have examined the mechanism by which the AhR regulates gene expression, few have been examined the regulation of expression of the AhR gene itself. The promoter region of the murine AhR gene has been cloned and sequenced and found to contain several SP1 binding sites, typical of TATA-less promoters. Here we describe experiments in which we have extended these studies and have analyzed a significantly larger fragment of 5'-flanking region of the mouse AhR gene. Interestingly, sequence analysis of 1500bp of the upstream region has revealed the presence of DNA binding sites for several major transcription factors, including: SP1, AP1, AP2, NF1, HNF1, CCAAT boxes, CRE and other sites. Interestingly, the 5' untranslated region of the gene contains a perfect dioxin responsive element and two metal-responsive elements. Deletion analysis experiments have been carried out to define the functionality of these elements. In addition to characterization of the upstream region, we have utilized an AhR gene promoter-luciferase reporter construct to determined that the low level of AhR gene expression observed in a variant mouse hepatoma (Hepa1c1c7) cell line (Tao) is not due to the loss of transcription factors necessary for efficient promoter function. In these experiments, expression of luciferase activity from the AhR gene promoter was comparable in wild-type and AhR-defective mouse cells.

1684 DNA DAMAGE, gadd-153 EXPRESSION, AND CYTOTOXICITY IN RENAL PROXIMAL TUBULAR EPITHELIAL CELLS

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We have previously shown that incubation of renal proximal tubular epithelial cells (LLC-PK_1) with 2-Br-*bis*-(glutathion-*S*-yl)hydroquinone (2-Br-*bis*-[GSyl]HQ) results in the generation of reactive oxygen species, DNA damage, and cytotoxicity. The present studies were initiated to determine the relationship between these events. 2-Br-*bis*-(GSyl)HQ caused concentration and time-dependent increases in DNA single strand breaks (DSSB), the expression of *gadd 153* (a gene inducible by growth arrest and DNA damage) and cytotoxicity. The iron chelator deferoxamine, and catalase prevented 2-Br-*bis*-(GSyl)-HQ-mediated DSSB, *gadd153* induction, and cytotoxicity. In contrast neither 1, 10-phenanthroline nor the copper ion chelator bathocuproine, prevented 2-Br-*bis*-(GSyl)HQ-mediated DSSB, *gadd153* induction or cytotoxicity. The endonuclease inhibitor aurintricarboxylic acid did not prevent DSSB or cytotoxicity. No significant further increase in DSSB was observed when LLC-PK_1 cells were incubated with 2-Br-*bis*-(GSyl)HQ in the presence of the DNA-repair inhibitors 1-β-D-arabinofuranosyl-cytosine and hydroxyurea, and the repair was complete by 1 hr. This indicates DNA repair similar to that observed after X-rays. The data suggest that 2-Br-*bis*-(GSyl)HQ-induced DSSB, and cell death, are dependent on Fe^{2+} and H_2O_2, and that these effects appear to be causally related. However, whether DNA damage directly serves as the signal for *gadd 153* induction or whether *gadd 153* expression is a consequence of growth arrest is not yet known. (ES 07359).

1685 STRUCTURAL ORGANIZATION AND POLYADENYLATION SITE ANALYSIS OF THE HUMAN PON GENE ENCODING SERUM PARAOXONASE/ ARYLESTERASE

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World Health Organization estimates suggests that there are as many as 3 million acute pesticide poisonings per year, with 220,000 deaths world wide. Serum paraoxonase (POase) is an 'A' esterase found associated with the high density lipoprotein fraction of mammalian serum. The enzyme hydrolyzes the active intermediates (oxons) of several organophosphate insecticides including chlorpyrifos, parathion, and diazinon, as well as the P-F bond of the nerve agents soman and sarin. We have shown previously that POase provides *in vivo* protection against OP toxicity. We have isolated fragments of the PON gene from lambda genomic libraries and other fragments from yeast artificial chromosomes carrying PON. These fragments form a 28 kb contig encompassing 300 bp of 5' sequence, the entire coding region, and 2kb of 3' flanking sequence of the PON gene. The structural portion of PON is encoded by 9 exons, that form the primary transcript by the use of typical splice donor and acceptor sites. PON messages previously examined were polyadenylated using non-canonical polyadenylation signal sequences. The 3' untranslated region surrounding and including the polyadenylation signal sequences in four individuals expressing different amounts of PON was amplified by PCR. Sequence analysis of the cloned PCR products revealed several apparent polymorphisms. These studies provide the groundwork for understanding the genetic factors controlling paraoxonase enzyme levels. (Supported by NIH Grant ES-05194)

1686 ROLE OF INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) IN C57B1/6 MICE EXPOSED TO SILICA PARTICLES

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ICAM-1, an adhesion molecule expressed on endothelial cells, participates in the migration of neutrophils (PMN) out of the blood and into the tissue parenchyma. In some models of lung injury, PMNs bind to ICAM-1 through the expression and activation of β2 integrins, Mac-1 and LFA-1, on the PMN cell membrane. Much research on this interaction has focused on the role of ICAM-1 in antigen-dependent models of lung injury. Although the pathogenic mechanisms of silica induced lung injury are well described, the role of ICAM-1 in lung injury elicited by this inorganic particle is unknown. The aim of this work is to examine the role of ICAM-1 in the inflammatory process elicited by the injection of silica into the lungs of mice. Animals received 3 mg/kg intraperitoneally of a rat monoclonal antibody (YN1/1.7.4) directed against mouse ICAM-1 1h prior to and 6 and 12h after an intratracheal injection of silica (2 mg/mouse). Animals were then sacrificed 24h later. The administration of anti-ICAM-1 antibody was shown to reduce levels of ICAM-1 protein by 46% in the lung as measured by an ELISA of lung tissue protein. PMN influx measured in the bronchoalveolar lavage was also found to be significantly reduced by 52% in mice exposed to silica. However, lung damage (protein and albumin in the lavage), soluble ICAM-1 levels in the lavage, and increased collagen deposition (lung hydroxyproline content measured at 14 days) were not reduced by anti-ICAM-1 administration. These data indicate that ICAM-1, in part, is important in the migration of PMNs into the lungs following silica exposure. However, it appears that PMN influx is both dependent and independent of elevated ICAM-1 expression in the lungs. Supported by UCONN Toxicology Program, AFPE, and UCONN Research Foundation.

1687 CHARACTERIZATION OF POLYMORPHISMS IN THE 5' UPSTREAM REGION OF THE HUMAN MICROSOMAL EPOXIDE HYDROLASE GENE

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Microsomal epoxide hydrolase (mEH) is an important xenobiotic metabolizing enzyme which, in most cases, acts to protect cells against highly reactive and cytotoxic electrophilic epoxides. Wide variations in human mEH enzyme activity have been observed in different cell types and between individuals within the population. We hypothesize that some of these activity differences may be explained by genetic polymorphisms present in the 5' upstream regulatory region of the gene. Single strand conformational polymorphism analysis was used to screen the region from +100 to -700 for the presence of genetic variations. Seven polymorphic sites were subsequently determined by DNA sequencing. To ascertain the functional significance of the individual nucleotide substitutions at these sites, DNA fragments containing the respective modifications were cloned into heterologous TK/CAT expression vectors and transfected into HepG2 cells. Results indicated that certain of these polymorphic loci may differentially regulate transcriptional activity. Allelic frequencies and linkages of these sites were determined by restriction fragment length polymorphism or competitive oligonucleotide priming assays. Further experiments are in progress to assess these polymorphisms more definitively and determine the molecular basis for their modulation of transcriptional activity. Supported by NIH grants ES04978 and ES07032.

1688 BLEOMYCIN INDUCED ALTERATIONS IN THE PULMONARY EPITHELIUM OF FIBROSIS SENSITIVE AND RESISTANT MICE

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Clinical use of the antineoplastic agent bleomycin is restricted due to pulmonary toxicity. A single intratracheally instilled dose of bleomycin has been used to produce pulmonary fibrosis in experimental mouse models which differ in suscepibility to bleomycin induced fibrosis. Previous studies have indicated that epithelial cells play a role in bleomycin induced injury. Both clara cell protein (CC10) and surfactant protein C (SPC) have been used as cell specific markers for bronchiolar epithelial clara cells and alveolar type II cells, respectively. In this study two strains of mice were treated with a single intratracheal dose of bleomycin and the pattern of SPC and CC10 mRNA expression was examined by *in situ* hybridization. Increased SPC mRNA abundance per cell was observed in both fibrosis resistant and sensitive strains of mice. In addition, SPC mRNA was detected in cells of the bronchiolar epithelium in the fibrosis sensitive mice. The pattern of expression of CC10 mRNA in the bronchiolar epithelium was also altered. In both mouse strains there was a decrease in the number of bronchiolar cells expressing CC10 mRNA although this decrease was more pronounced in the fibrosis sensitive mice. The strain dependent differences in epithelial gene expression suggest a role for the epithelium in pulmonary repair or fibrosis. Supported by CA27791, HL36543, ES07026.

1689 ANALYSIS OF THE 5'UTR OF AN NMDA RECEPTOR SUBUNIT SUGGESTS CONTROLLED TRANSLATIONAL EFFICIENCY

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Within the central nervous system, glutamate is the primary mediator of fast excitatory neurotransmission. Of the class of ligand gated ion channels responsive to glutamate, the N-methyl-D-aspartate (NMDA) receptor possesses a uniquely high calcium permeability. This property of the receptor defines its role in the phenomenon of excitotoxicity. In this model, excessive glutamate release causes extreme NMDA receptor activation and subsequent neurodegeneration. Thus, a balance between cellular function and survival suggests that neurons regulate the amount of NMDA receptor protein they present to cell surfaces. DNA transcription is the principal mechanism for control of protein expression, although messenger RNA (mRNA) translation may also be regulated. The NR1 subunit of the NMDA receptor is ubiquitously transcribed throughout the brain. *Xenopus laevis* oocytes injected with NR1 mRNA form functional channels with small agonist responses. NR2 subunits exhibit controlled transcription and form functional receptors only when coinjected with NR1. In *Xenopus oocytes*, the 5' untranslated region (5'UTR) of the mouse NR2A subunit is capable of restricting protein translation to 1% of maximum, thereby controlling NMDA receptor activity over three orders of magnitude. Analysis of this translational inhibition reveals little contribution from the five alternate start codons contained within the 5'UTR. Serial truncations of the 5'UTR implicate secondary structure as the prime inhibitor. No translational inhibition is exhibited by the 5'UTR of the NR1 subunit. Across rat and mouse species, greater than 99% sequence identity exists within the 5'UTR of NR2A, with only 95% present in the coding sequences. Furthermore, rat/mouse sequence comparisons of NR1 contrast those of NR2A (i.e., 88%) in the 5'UTR, 96% in the coding region). Taken together, these findings suggest a conserved mechanism for control of NMDA receptor activity via the modulation of translational efficiency of a single subunit.

1690 ANALYSIS OF THE 5' FLANKING REGION OF THE RABBIT FLAVIN-CONTAINING MONOOXYGENASE FORM 2 (FMO2) GENE

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Microsomal flavin-containing monooxygenases (FMO) represent a family of differentially expressed xenobiotic-metabolizing enzymes. Distinct tissue-specific patterns of expression for one isoform, FMO2 have been characterized by this and other laboratories. In order to determine mechanisms controlling FMO2 gene expression, we isolated and have begun characterization of FMO2 genomic clones. By screening a rabbit liver λEMBL3 genomic library with the cDNA for FMO2, we isolated two positive clones which span approximately 30 kb. The genomic clones were mapped with restriction endonucleases and subcloned for more detailed mapping and sequence analysis. The clones contain approximately 800 bp of coding sequence, including the transcription start site and 3.5 kb of 5' flanking region. Using the primer extension method, we have determined the transcription start site of the gene, which is followed by a 57 bp non-coding exon 1. Sequence analysis also revealed a 369 bp intron 1. The second exon is 172 bp and contains the translation start codon 41 bp from its 5' end. The transcription start site sequences are not representative of a classical TATA promoter, but do share homology with Inr promoter sequences. By isolating the cells from rabbit lung which express FMO2, and preparing nuclei and DNA from them, it was possible to evaluate DNase I hypersensitive and methylated sites, and compare these sites to those in liver, a tissue that does not express FMO2. No difference in the methylation status was observed, however, there are DNase I hypersensitive sites in the rabbit lung FMO2 gene that are not found in liver. One prominent hypersensitive site is localized approximately 40 bp

upstream from the transcription start site, near a PEA3 DNA-binding motif. Taken together, it is likely that tissue-specific transcription factors play a more prominent role in regulating FMO2 expression than methylation.

1691 DNA DAMAGE-INDUCIBLE GENES AS BIOMARKERS FOR LOW-DOSE EXPOSURES TO IONIZING RADIATION AND CHEMICAL GENOTOXICANTS

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A wide variety of DNA damaging agents have been shown to induce the expression of genes including p53, Cip1, Gadd 45 and Gadd 153. The purpose of this research was to determine whether the expression of these genes can act as a sensitive biomarker of low-dose exposure to ionizing radiation or chemicals. Rodent (LEC) and human cell lines (A549) were exposed to X rays, α-particles, 4-nitro-quinoline-1-oxide (4NQO), or methyl methanesulfonate (MMS) and the levels of p53, Cip1, Gadd 45, and Gadd 153 proteins were determined by flow cytometry using indirect antibody labeling. These studies showed that the change in levels of DNA damage-inducible genes is an extremely sensitive indicator of exposure. The levels of p53 protein were altered significantly at a dose of 6 mGy of α-particle radiation and 10 ng/ml of 4NQO. Ionizing radiation and 4NQO readily induced p53 and Cip1 but not Gadd 153. MMS exposure (10 μg/ml) resulted in elevated levels of Gadd 153 but not p53 or Cip1. The temporal expression of DNA damage-inducible genes also differed among the exposure agents. These results show that DNA damage-inducible genes can act as sensitive biomarkers of exposure. [Research sponsored by U.S. OHER/DOE under Contract No. DE-AC04-76EVO1013.]

1692 HALOTHANE DECREASES THE ACTIVITY OF UDP-GLUCOSE:GLYCOPROTEIN GLUCOSYLTRANSFERASE, A LUMINAL ENDOPLASMIC RETICULUM PROTEIN INVOLVED IN PROTEIN FOLDING

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Covalent modification of liver proteins by the reactive trifluoroacetyl (TFA) chloride metabolite of halothane is thought to lead to an immune response in halothane hepatitis patients. However, it is not known how the immune system is activated. To understand the mechanism of immune activation, we have studied the processing of the TFA-proteins in rat hepatocytes. During these studies, we found a TFA-labeled 170 kDa protein (TFA-170) that co-precipitated with the 57, 58, 59 and 82 kDa protein targets of the TFA chloride metabolite when hepatocyte lysates were immunoprecipitated with protein-specific antisera. The TFA-170 was identified as UDP-glucose:glycoprotein glucosyl-transferase (UGGT), a luminal endoplasmic reticulum protein that adds glucose to high mannose glycoproteins and is thought to be involved in protein folding. The activity of UGGT was reduced in the microsomes of halothane-treated rats as compared to that of the control rats. This reduction in activity corresponded to a decrease in the amount of protein as evidenced by immunoblotting of rat microsomes with UGGT antiserum. These findings indicate that halothane may affect protein folding by decreasing the level of UGGT. This effect may be important in the etiology of halothane hepatitis.

1693 IN VIVO AND IN VITRO BINDING OF MICROCYSTIN TO PROTEIN PHOSPHATASES 1 AND 2A

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The hepatotoxic microcystins (Mcyst) are cyclic heptapeptides produced by cyanobacteria that are potent and specific inhibitors of the ser/thr protein phosphatases (PP1 and PP2A) with IC_{50}'s of 0.1–1.0 nM. Mcyst and other PP inhibitors like okadaic acid or calyculin A interact with the C-terminal region of the catalytic subunit of PP1 and PP2A. Using ^{125}I-Mcyst and antibodies specific for PP1 and PP2A, we show by immunoprecipitation and autoradiography, that in hepatocytes Mcyst forms secondary covalent bonds with both PP1 and PP2A catalytic subunits. We demonstrate that the bond resulted from the reaction between the electrophilic α, β unsaturated carbonyl of the methyldehydroalanine residue of Mcyst and the thiol of Cys 273 located in the C-terminal of PP1 (Cys 266 in PP2A), since site-directed mutagenesis of Cys 273 to Ala in PP1α led to complete loss of ability for the formation of a covalent Mcyst-PP1 adduct. This covalent binding explains the persistence of Mcyst in liver. It also explains the potency of Mcyst as tumor promoter since even small increases in the level of protein phosphorylation in cells resulting from partial PP inhibition with concomitant increases in kinase activity, will lead, over time, to profound changes in the control of cell growth and differentiation. (Support NIEHS Grant ESO5678 to MR, NIH grant DK18512 to EYCL, and NIH CA54167 and TRDRP 3KT-0193 to NB).

1694 SYNERGISTIC EFFECT OF THE BENZENE METABOLITE HYDROQUINONE ON 12-O-TETRADECANOYL PHORBOL-13-ACETATE (TPA)- INDUCED HUMAN MYELOBLASTIC LEUKEMIA CELL DIFFERENTIATION

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The purpose of this study was to examine the dose response interaction between TPA and hydroquinone (HQ) on the differentiation of ML-1 cells. Upon differentiation these cells exhibit a phenotype typical of monocytes/macrophages after a 3 day treatment with 0.3 ng/ml TPA followed by 3 days in culture without TPA. Indicative of this phenotype, these cells exhibit mitochondrial respiration with accompanying superoxide generation. Treatment with 0.15 ng/ml TPA results in partially differentiated cells which display very low mitochondrial respiration while treatment with 1.2 ng/ml TPA produces cells with a dendritic-like morphology and an absence of mitochondrial superoxide production. Co-treatment with 5 μM HQ and 0.15 ng/ml TPA results in cells which exhibit mitochondrial respiration similar to that of cells induced to differentiate with 0.3 ng/ml TPA. In contrast, co-treatment with 5 μM HQ and 0.3 ng/ml TPA results in arrested cell differentiation. These cells are biochemically and morphologically similar to those treated with 1.2 ng/ml TPA. These findings suggest that the effects of HQ on ML-1 cell differentiation result from a synergism with TPA. A possible mechanism by which HQ synergizes with TPA may be through alternations in the cysteine-rich region in the regulatory domain of PKC, a receptor for TPA, resulting in enhanced signal transduction and gene expression during ML-1 cell differentiation. Supported by ES03760, ES07141 and CAAT.

1695 MOLECULAR GENETIC ANALYSIS OF PORPHYRIA INDUCED BY IRON AND HEXACHLOROBENZENE IN C57BL/10ScSn X DBA/2 F_2 MICE

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The induction of porphyria in mice by hexachlorobenzene (HCB) together with iron, models the disease in humans caused by HCB and also sporadic porphyria cutanea tarda. The Ah^{b-1} strain C57BL/10ScSn is susceptible whereas the Ah^{d} strain DBA/2 is not. The genetic predisposition has been studied in an F_2 intercross of these prototype strains. Two hundred F_2 mice received a single s.c. dose of iron-dextran (600 mg Fe/kg) and HCB in their diet (0.02%) for 7 weeks. The highest and lowest responding mice were selected for molecular genetic analysis. Regions of chromosomes 14 and 17 were found to be strongly linked with development of porphyria after genotyping the two groups with DNA microsatellite markers by PCR. Correlation of the Ah^{b-1} allele (chromosome 12) with porphyria was demonstrated with a restriction fragment length polymorphism in the Ah receptor gene. However, mRNA levels of *Cyp1A2* were detectable in both high and low responding groups. Some correspondence was found with mRNA for *Cyp1a1*. Hepatic microsomal EROD activities showed a 2-fold increase in the high over the low group. MROD, BROD and PROD activities were comparable between groups.

1696 GENE TARGETING AT THE *AHR* LOCUS

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2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related halogenated aromatic hydrocarbons are major environmental contaminants, and TCDD is a tumor promoter and teratogen in animal models. The effects of TCDD are

mediated by binding to a cytosolic protein named the Ah receptor (AHR). Gene targeting of the AHR basic/helix-loop-helix domain was used to inactivate the murine *Ahr* gene by homologous recombination in embryonic stem cells. *Ahr*$^{-/-}$ mice were recovered in the expected Mendelian ratio at two weeks of age, with no evidence of prenatal or perinatal lethality. Western blot of liver cytosol from these mice detects no AHR protein, and the animals are resistant to TCDD induction of CYPIA1. At one week of age the *Ahr*$^{-/-}$ mice display a hepatic phenotype including decreased liver weight, severe fatty metamorphosis and persistent extramedullary hematopoiesis. This phenotype resolves within one to two weeks. At two weeks of age and beyond the *Ahr*$^{-/-}$ mice show decreased liver weight and portal fibrosis. This *Ahr* null mouse line indicates a role for the AHR in liver growth or maturation, and future experiments will be aimed at understanding the endogenous role of this protein. These animals may be important in risk analysis because they will allow the delineation of receptor and non-receptor-mediated effects of TCDD and other agonists.

1697 EVIDENCE OF DNA HYPOMETHYLATION DUE TO BROMOBENZENE-INDUCED GLUTATHIONE/ METHIONINE DEPLETION

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We have shown in a recent study that there is a tight interrelationship between liver glutathione (GSH) and methionine (Met), and that depletion of GSH leads to Met depletion and impairs methylation. Dietary deficiency of methyl-donors is known to cause liver tumor in rodents, and DNA hypomethylation has been postulated as the cause of neoplastic lesions. We had previously shown that liver necrosis caused by a high dose of bromobenzene (BB), a GSH depleting agent, is associated with an impairment in the methylation of bromothiocatechols and bromodihydrobenzenethiolols. The aim of the present study was to examine the effect of BB toxicity on the methylation of DNA. A single ip dose of Met (450 mg/kg) labeled with [$^{14}CH_3$]Met (5 μCi) given at 5.5 hr after BB (800 mg/kg) significantly protected the liver against necrosis in the Syrian hamster. The protection was accompanied with a significant incorporation of [$^{14}CH_3$] into liver DNA 24 h after BB. Approximately a 5-fold increased in the incorporation was found in the BB + [$^{14}CH_3$]Met treated hamsters compared with hamsters that received only [$^{14}CH_3$]Met. These results indicated that BB toxicity is associated with an impairment in DNA methylation; impairment in methylation occurs as a result of GSH/Met depletion. DNA hypomethylation could be the initial factor that elicits toxicity in response to a variety of GSH depleting agents including those modeled here by BB.

1698 STRESS GENE AND GROWTH RELATED GENE EXPRESSION FOLLOWING ACUTE RENAL INJURY

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Although hydroquinone (HQ) is nephrocarcinogenic in the male Fischer 344 rat, it is generally non-mutagenic in short term bioassays. It has been proposed that alterations in gene expression and growth control play a role in renal carcino-genesis. We therefore measured stress-response (*grp 78* and *hsp 70*) and growth-related (*gadd 153* and *histone H2B*) gene-expression during acute renal injury and subsequent regeneration following administration of HQ (4.5 mmol/kg, po) or 2, 3, 5-(tri-glutathion-*S*-yl)HQ (7.5 μmol/kg, iv) to male F344 rats. Both compounds caused a marked time dependant (2–5 h) increase in *grp 78* and *hsp 70* mRNA levels, which correlated with the progression of toxicity, with levels returning to control values 24–96 h after dosing. *Gadd 153* mRNA (a gene inducible by growth arrest and DNA damage) was maximal 5 h after dosing. Concomitant with the marked increase in *gadd 153* mRNA, *histone H2B* mRNA was decreased to 60% of controls 1–5 h after dosing, consistent with histone protein synthesis being tightly coupled to DNA synthesis. Thus, *H2B* mRNA was elevated 4-fold between 24–96 h after dosing, coincident with maximal cell regeneration, as evidenced by the incorporation of bromodeoxyuridine into newly synthesized DNA. The similar alternations in gene-expression and injury caused by HQ and 2, 3, 5-(tri-glutathion-*S*-yl)HQ indicate a role for the latter in HQ-mediated nephro-toxicity. The mechanism by which such changes in gene-expression contribute to HQ-mediated nephrocarcinogenicity requires further investigation (GM 39338).

1699 COVALENT AND OXIDATIVE PROTEIN MODIFICATION BY NEPHROTOXIC QUINONE-THIOETHERS IN VIVO

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Administration of quinone-thioethers to rats results in severe renal proximal tubular necrosis. Because the reactivity of quinones lies in their ability to redox cycle and/or to alkylate proteins, we sought to determine the role of covalent binding and oxidative protein damage in quinone-thioether mediated nephrotoxicity. To detect covalent protein adducts, antibodies were generated using 2-bromo-6-(*N*-acetylcystein-*S*-yl) hydro-quinone-keyhole-limpet hemocyanin (2-BrHQ-NAC-KLH) as an immunogen. Specificity towards the 2-BrHQ-NAC epitope has been demonstrated previously. Antibodies recognized covalently modified proteins at nephrotoxic doses of 2-BrHQ, 2-Br-*bis*-(glutathion-*S*-yl)HQ (2-Br-*bis*--[GSyl]HQ), HQ, and HQ-GSH conjugates, in male Fischer 344 rat kidneys, but not in untreated rat kidneys. Western blot analysis of cytosol revealed four predominant immunostaining proteins at approximately 33, 34, 43, and 87 kDa, with several proteins exhibiting lighter staining between 45 and 85 kDa. To assess oxidative protein damage, male F344 rats were dosed with 2-Br-*bis*-(GSyl)HQ (30 μmol/kg, *iv*). There were significant time-dependent elevations in renal protein carbonyls, with maximal increases reaching 127% of control values 8 hr after dosing. There were no significant elevations in protein carbonyls in liver, a non-target organ. The data indicate that quinone-thioethers produce both covalent and oxidative protein damage in target tissue. The relative importance of these modifications in quinone-thioether-mediated toxicity remains to be determined. (ES 07359, GM 39338).

1700 MOLECULAR FINGERPRINTING OF NSG-PEPTOIDS WITH ANTI-BACTERIAL ACTIVITY

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Simon et al., (1992) have previously described the synthesis of a new class of biopolymer containing non-natural backbones (oligo-N-substituted glycines, NSG-peptoids). Two peptoids (CHIR10428 & CHIR10429) were identified and characterized on the basis of their cytotoxic/cytostatic activity on gram negative bacterial targets. To gain some insight into the mechanism of their anti-bacterial and potential antineoplastic effect, CHIR10428 and CHIR10429 were tested at sub-cytotoxic doses (EC_{50}) using a reporter gene assay (Pro-Tox, Xenometrix). The stress genes induced after exposure to these peptoids included *clpB* and *micF* (indicating protein and membrane damage), as well as *osmY* (indicating changes in cellular osmolarity). The time-dependent sequence of gene induction for CHIR10428 (*clpB/micF* −30 min, *osmY* −45 min, *umuDC* −60 min) was compared to the gene expression kinetics of bleomycin (*nfo, clpB, dinD, umuDC* −15 min) and mitomycin C (*dinD, recA, umuDC* − 15 min). These data suggest that the antibacterial mechanisms of toxicity/action of CHIR10428 are different than bleomycin and mitomycin C.

1701 MICROSATELLITE ALTERATIONS IN B6C3F1 MOUSE LIVER TUMORS

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Microsatellite sequence length alterations can be an indicative marker for genomic instability. Frequently microsatellite changes have been detected in certain heritable forms of human colon cancer. These changes were shown to be the direct consequence of mutations in DNA mismatch repair genes, which are partly responsible for maintaining the sequence integrity of the genome. Given that the B6C3F1 mouse is genetically susceptible for the development of liver tumors we determined whether tumors from these mice contained microsatellite sequence alterations. Analysis of 48 tumors at 24 different microsatellite loci revealed microsatellite alterations in 20 of the 48 tumors (42%). This frequency is relatively high suggesting that the microsatellite alterations may be due to general genetic instability. However, only a single tumor contained multiple sequence alterations and microsatellite alterations were detected in 27% of the DNA derived from normal liver tissue. Therefore, we conclude that the microsatellite changes detected in the liver tumors were due to spontaneous mutations occurring prior to tumor development. This suggests that the genetic instability operational in the development of some forms of hereditary human colon cancers does not play

a role in the genetic predisposition to develop liver tumors in the B6C3F1 mouse. Additionally, we demonstrate that H-ras gene activation, which causes some forms of genetic instability in vitro, is not associated with genetic instability in these tumors as measured by microsatellite alterations.

1702 ATTENUATION OF CELL CYCLE CHECKPOINT RESPONSES TO DNA DAMAGE IS ASSOCIATED WITH IMMORTALIZATION IN MOUSE HEPATOCYTES

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Genomic integrity is maintained in part by a vast array of cellular checkpoint controls that can sense and respond appropriately to DNA alterations. Typical cell cycle checkpoint responses to DNA damage include cell cycle delay or arrest, DNA repair and/or cell death. Extensive work in this area has suggested that loss of checkpoint controls may play an important role in the evolution of cancer. In trying to elucidate possible mechanisms involved in mouse liver tumor formation, studies were initiated to investigate potential alterations in checkpoint controls during hepatocellular transformation. Normal checkpoint responses to DNA damage were characterized in primary cultures of B6C3F1 mouse hepatocytes and compared to those seen with spontaneously immortalized, preneoplastic hepatocyte cell lines. To induce DNA damage, cells were exposed to the radiomimetic drug, bleomycin (8–50 μg/ml). Cell cycle progression was assessed by flow cytometry, and cell death was monitored by cell counts. Primary cultures of B6C3F1 mouse hepatocytes responded to DNA damage by arresting in G1 or G2 and by undergoing significant cell death. The immortalized mouse hepatocyte cell lines did not arrest in G1, but accumulated in G2. Very little cell death was observed with these cells. These results suggest that attenuation of G1 and cell death checkpoints may be early changes that occur during cellular transformation of mouse hepatocytes.

1703 RT-PCR ANALYSIS OF THE EXPRESSION OF ORGANIC ANION TRANSPORTING POLYPEPTIDE MESSENGER RNA

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The hepatic organic anion transporting polypeptide (oatp) mediates sodium-independent uptake of monovalent (e.g., bile acids) and divalent (e.g., sulfobromophthalein) cholephilic organic anions. Oatp is believed to be important for drug clearance by the liver and kidney. Oatp or related peptides may serve unknown functions in other tissues. In Northern blot analyses, the cDNA for oatp reacted with multiple mRNAs of rat liver, kidney, brain, lung, skeletal muscle and colon (Jacquemin, et al. PNAS 91:133, 1994). Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) offers increased specificity and sensitivity over other methods of analysis to determine the level of expression of oatp mRNA. The levels of oatp mRNA (relative to β-actin mRNA) were assayed in rat liver, kidney, brain and lung using RT-PCR to define tissues where oatp mRNA is specifically expressed. Specific PCR conditions for oatp mRNA were established which show a linear response with input template concentration and highly reproducible values of replicate samples. Rat liver contains more than ten-fold greater oatp mRNA than kidney and two-fold greater oatp mRNA than brain and lung. These experiments establish PCR assay conditions for future studies of the modulation of oatp gene expression. (Supported by NIH Grant ES-03192)

1704 MOLECULAR AND CELLULAR TARGETS OF BENZO(A)PYRENE IN PRECISION-CUT RAT LIVER AND KIDNEY SLICES

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Benzo(a)pyrene (BaP) and related aromatic hydrocarbons (AHs) interfere with growth-related signaling in liver and kidney following acute or repeated challenge *in vivo* and *in vitro*. Of particular significance has been the finding that distinct cell populations within these organs are not uniformly sensitive to AHs. Taking advantage of the preservation of normal organ architecture in precision-cut tissue slices, the current studies were designed to further characterize the cellular and molecular targets of BaP within liver and kidney. Precision-cut liver and kidney slices harvested from naive, adult female Sprague-Dawley rats were challenged with various concentrations of BaP (0.3–30 μM) for 0.5–24 hr and processed for measurements of growth-related gene expression by Northern analysis. ATP and K^+ contents were monitored as indices of cellular viability. Northern analysis revealed decreased expression of the c-jun proto-oncogene in kidney, but not liver slices, following incubation with 30 μM BaP. BaP did not reduce ATP content in liver or kidney slices at any of the concentrations or time-points examined. These data demonstrate that BaP alters growth-related proto-oncogene expression in renal slices and that this response does not represent generalized cytotoxicity. [Supported by NIH grant ES 04917 and 00213 (KSR) and ES 05790 (KB)].

1705 MOLECULAR TARGETS OF BENZO(A)PYRENE IN VASCULAR SMOOTH MUSCLE CELLS (SMCs)

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The critical target genes of BaP-induced atherogenesis remain to be identified. A role for ras genes is suggested by evidence that BaP upregulates ras gene expression in cultured SMCs, and that inhibition of ras function prevents SMC proliferation following vascular injury *in vivo*. In the present study, we have investigated the role of activating ras mutations, alternative RNA splicing of Ha-ras, and differential gene expression in BaP-treated SMCs. Aortic SMCs challenged with 3 μM BaP for 24 hr and subcultured for nine passages were screened by reverse transcription-polymerase chain reaction (RT-PCR) for mutations in exons 1 and 2 of Ha-ras, N-ras, and Ki-ras genes. No mutations were detected in the regions examined. The size of c-Ha-ras transcripts was the same in control and BaP-treated cells suggesting that splicing of the alternative exon IDX was unchanged in cells treated with 3 μM BaP for 6 hr. To identify immediate gene targets of BaP, aortic SMC cultures were treated with 3 μM BaP for 6 hr and processed for differential display PCR. BaP augmented transcription of 5 discrete PCR products and decreased 4 others. These data suggest that activating ras mutations or alternative IDX splicing are not critical for BaP-induced alternations of ras function. Identification of genes differentially expressed in BaP-treated SMCs will better define the molecular basis of the atherogenic response. (Supported by NIH grant ES 04949 and 00213).

1706 EFFECT OF ACRYLONITRILE (VCN) ON REACTIVE OXYGEN SPECIES MEDIATED STRAND BREAKS IN pBLUESCRIPT PLASMID DNA IN VITRO

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Studies in our laboratory indicated that VCN is oxidized to reactive intermediates that release cyanide (CN), by reactive oxygen species (ROS) generating systems *in vitro* and under cellular conditions. As part of our ongoing research to elucidate the mechanism of VCN induced genotoxicity, we aimed in the current study to investigate the types and rates of reaction of VCN or its metabolites with pBluescript supercoiled plasmid DNA. We utilized Fenton-like reaction or Hypoxanthine / Xanthine oxidase system as source for ROS. In presence of ROS, VCN activation occurred and CN release was quantitated. When VCN, alone, was incubated with plasmid DNA, no damage was observed. When ROS generating system, alone, was incubated with plasmid DNA, supercoiled plasmid DNA was converted to circular and/or linear form depending on ROS concentration. When VCN was added to the previous mixture the rate of ROS-mediated damage of supercoiled DNA was significantly reduced suggesting that VCN may act as a scavenger to ROS. The effects of the antioxidant glutathione (GSH) and the hydroxyl radical scavenger Ethanol (EtOH) on the rate of plasmid DNA damage in presence or absence of VCN were studied. GSH, (10 & 100 μM) enhanced ROS-induced DNA strand breaks. VCN, induced a concentration dependent inhibition of GSH enhancement of ROS formation and DNA strand breaks. GSH, however, induced an increase in levels of CN release from VCN. Addition of ethanol (1 & 10 %) reduced ROS formation, plasmid DNA strand breaks and decreased levels of CN production. This data support a mechanism of VCN oxidation by free radical and suggest an inhibitory effect of VCN on ROS-induced strand breaks in pBluescript plasmid DNA *in vitro*. [Supported by NIH grant # ES 01871].

1707 EFFECT OF ACROLEIN EXPOSURE ON ANGIOTENSIN-II RECEPTOR SUBTYPE IN PULMONARY ARTERY ENDOTHELIAL CELLS

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Angiotensin-II (A-II) plays a critical role in regulation of vascular tone. Because exposure to acrolein (ACR) results in plasma membrane protein sulfhydryl (SH) modulation in pulmonary artery endothelial cells (PAEC), we sought to determine the effect of ACR on A-II receptor subtype AT_1 and AT_2 in the presence and absence of ACR-scavenging reagents. PAEC were incubated in Hanks' balanced salt solution (HBSS) containing 1.0 and 1.5 μM ACR with and without the presence of 0.5 mM each of glutathione (GSH) or N-acetyl-L-cysteine (NAC) at 37°C for 30 min. Controls were incubated in HBSS alone or HBSS containing GSH or NAC under identical conditions. After incubation cells were washed and specific binding of ^{125}I-labeled A-II was measured with or without the presence of 10 μM each of the [Sar^1 Ile^8]-A-II, DuP 753, or PD 12317 (antagonist for total A-II, AT_1 and AT_2, respectively) at 25°C for 45 min. Non-specific binding was measured in the presence of 10 μM unlabeled A-II. In control PAEC total A-II, AT_1, and AT_2 binding was 20.6 ± 2.1, 14.4 ± 1.8, and 7.1 ± 1.0 fmol/mg protein, respectively. Exposure of PAEC to 1 μM or 1.5 μM ACR significantly reduced total and AT_1-specific but not AT_2-specific binding ($p < 0.05$ for both total and AT_1 versus controls and for both concentrations of ACR). Incubation of PAEC in the presence of 0.5 mM GSH or NAC prevented ACR (1.0 or 1.5 μM)-induced reduction of total A-II- and AT_1-specific receptor binding. These results demonstrate that ACR exposure is responsible for reduction of total A-II receptor binding and that modulation of SH status of AT_1 receptors appears associated with reduction of A-II binding in PAEC.

1708 SPECIES SPECIFICITY FOR GASTRIC GLAND ATROPHY INDUCED BY A GASTRIN RECEPTOR ANTAGONIST

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Oral administration of the gastrin antagonist CI-988 causes gastric gland degeneration and atrophy within 14 days when administered to cynomolgus monkeys at 100 mg/kg/day. In contrast, administration of CI-988 to rats at 3200 mg/kg/day or to mice at 2000 mg/kg/day fails to produce histopathologic effects in the stomach. Differences in the plasma drug concentrations of CI-988 in monkeys, rats, and mice were insufficient to suggest a toxicokinetic explanation for the species specifity for the gastrin antagonist induced gastric atrophy. For example, oral administration of CI-988 at 100 mg/kg/day was sufficient to induce mild gastric gland degeneration in monkeys within two weeks and was associated with Cmax and AUC values of 35 ng/mL and 145 ng/mL/hr, respectively. In contrast, CI-988 at 3200 mg/kg/day was associated with Cmax and AUC values of 34 ng/mL and 525 ng/mL/hr, respectively in rats, and failed to induce gastric gland changes. Further, CI-988 at 2000 mg/kg/day was associated with Cmax and AUC values of 369 ng/mL and 2168 ng/mL/hr, respectively in mice, yet gastric gland changes did not occur. Highly conserved gastrin receptor sequence homology reported in the literature suggest some other fundamental, but as yet undefined biochemical, cellular, or physiological differences accounting for CI-988's species specific effect on gastric mucosa.

1709 METABOLISM, TOXICITY AND INTERACTION WITH CELLULAR SECOND MESSAGE SYSTEMS OF THE LDL RECEPTOR GENE UPREGULATOR, CP-230,821

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CP-230,821 is a novel, potent upregulator of the LDL receptor gene. The structure contains a thiosemicarbazone function and when administered to rats and hamsters produced side effects consistent with thiourea exposure. Azo bond cleavage to produce a thiourea from the parent thiosemicabazone was investigated by characterization of the metabolism of CP-230,821 in the rat and in isolated rat liver microsomes. No thiourea-containing metabolites were found in either the in vivo or in vitro systems. Under reductive metabolism conditions in rat liver microsomes, no loss of parent compound was noted. Efforts to dissect the side effects away from the desired pharmacological effect examined possible interactions with cellular second message systems. Interactions with protein kinases were investigated by the use of relatively specific inhibitors of protein kinase C (H7) and tyrosine kinases (genestein). Pretreatment of the cells with H7 had no effect on CP-230,821-stimulated LDL receptor protein expression while there was a clear concentration-dependent inhibition of LDL receptor expression with genestein pretreatment. It was concluded that the in vivo side effects observed after treatment of rats with CP-230,821 was probably not due to the metabolism of the thiosemicarbazone to a thiourea. Rather, CP-230,821 may be acting at the cellular level through the stimulation of tyrosine kinase(s) and that this nonspecific interaction with cellular second message systems may be responsible for the in vivo side effects observed upon administration of CP-230,821.

1710 ALTERATIONS OF NUCLEAR TRANSCRIPTION FACTOR LEVELS FOLLOWING CCl_4 TOXICANT EXPOSURE

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Activation of NF-kB in regenerating rat liver has been demonstrated by Cressman *et al.*(J. Biol. Chem. 269, 30429, 1994). Knock out experiments in mice (Hilberg *et al.* Nature 365, 179, 1993; Beg *et al.* Nature 376, 167, 1995), have also demonstrated an essential role for NF-kB as well as c-Jun in liver proliferation. In view of these findings we examined the effects of CCl_4 on rat hepatic tissue nuclear levels of a number of transcription factors including NF-kB and c-jun(AP-1). Male Sprague Dawley Rats (120–130 gms) were injected interperitoneally with 160 mg/kg CCl_4 and sacrificed at various times post-treatment. Nuclear extracts prepared from the hepatic tissue were then monitored for levels of transcription factors as a function of post treatment times utilizing electrophoretic mobility gel shift assays (EMSA's). A striking increase in the levels of AP-1 at 2 through 24 hrs CCl_4 post-treatment as well as a significant increase in NF-kB levels at 2 through 24 hrs post-treatment was apparent. A smaller increase was observed in the levels of AP-3, as well as consistent small to moderate decreases in GRE, TFIID, and cMyc transcription factor levels. No apparent or significant changes were seen in the levels of the SP-1, Oct-1, CREB, CTF/NF-1 and AP-2 transcription factors. These results further support the view that both NF-kB and c-Jun may be required for the regulation of key genes involved in hepatocyte proliferation and regeneration. Supported by NIEHS grant ES02521.

1711 ALTERATION OF MAJOR CYTOKINES AFTER REPEATED ORAL EXPOSURES TO T-2 TOXIN IN CD-1 MICE

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Immunotoxicity of Fusarium T-2 toxin has been well documented. Male CD-1 mice were orally gavaged with 0, 0.1, 0.5, and 2.5 mg/kg of T-2 toxin on alternate days for two weeks. Two days after the last dose splenic non-adherent cells were cultured in the presence of concanavalin A to evaluate the status of interleukin (IL)-2, IL-3 and interferon (IFN)-γ. Additional groups of animals were injected ip with Brewer's thioglycolate broth; peritoneal macrophages were derived and cultured with lipopolysaccharide to measure the activities of IL-1α, IL-6, and tumor necrosis factor (TNF)-α. Cellular expression of each cytokine was evaluated by northern blots with appropriate riboprobes or by DNA amplification procedure, and the secreted cytokines were measured by ELISA. The mycotoxin exposure increased the IL-2, IL-3 and IFN-γ mRNA expression with concurrent increase in protein levels. Levels of IL-1α, IL-6 and TNF-α mRNA were not altered but their products were decreased in the medium and the number of recovered macrophages was greatly reduced at the highest dose. Systemic exposure to T-2 toxin thus selectively influences cytokine pathways, perhaps involving direct effects on cells and also via systemic endotoxemia associated with oral exposure to this toxin. The effects of T-2 on cytokines are complex and paradoxical.

1712 IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES DUE TO GLUCOCORTICOID ACTION IN A MOUSE FIBROBLAST CELL LINE

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The steroid hormone family of glucocorticoids are widely used therapeutically for treatment of allergic diseases such as asthma, autoimmune diseases, inflammatory disorders (rheumatoid arthritis), graft rejection, and as a chemotherapeutic agent. The physiological and therapeutic pharmacologic effects of glucocorticoids depend on ligand-receptor binding and receptor mediated changes in gene expression. Numerous experiments have been done with RNA and protein synthesis inhibitors showing that the rapid metabolic and

apoptotic responses due to glucocorticoids are most likely dependent on the induction of protein mediators. Overall, there has been an inability to identify specific, primary response mediators of the glucocorticoid-related metabolic and apoptotic responses. Our model system to identify candidate mediators consists of the transformed mouse fibroblast cell line ID13 which undergoes morphological changes and growth suppression after treatment with the glucocorticoid dexamethasone ($10^{-6}M$). By use of "giant" two-dimensional gel electrophoresis, labeled cellular proteins and *in vitro* translated mRNAs have revealed many changes in protein expression after dexamethasone treatment. In an attempt to clone these proteins, the molecular biological approaches of plus/minus screening and subtractive hybridization using an ID13 lambda phage library have been undertaken. Clones have been identified for metallothionein and G stimulatory protein alpha(Gs), two known dexamethasone induced genes. A further clone has been isolated that shows dexamethasone and cycloheximide induction by northern blot analysis. This clone was partially sequenced and comparison with the Gene Bank database found no genes with high homology. Further studies will investigate this gene and other clones as candidate mediators of glucocortiocoid effects. Supported by NIH DK16177

1713 REGULATION OF NEUROTROPHIN RECEPTOR TRK-B IN MANGANESE-INDUCED NEUROTOXICITY

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Environmental and occupational exposure of manganese (Mn) has been implicated in the etiology of Parkinson's disease. This study was undertaken to determine whether manganese exposure alters the regulation of endogenous neurotrophins, and their relationship to the underlying mechanisms of neruodegeneration. Mn (1 μmol in 5μl) was microinjected unilaterally into the striatum, and biochemical analyses were performed one week after the injection. Mn treatment produced a significant depletion of dopamine, DOPAC and HVA levels, and a significant elevation of malonaldehyde level in ipsilateral striatum as compared to contralateral. Immunohistochemical analysis of tyrosine kinase receptor Trk-B revealed a significant increase in the immunoreactivity at 24 hr after the treatment and a slight decrease at 7 days post treatment. This result suggests that exposure to manganese may alter the regulation of neurotrophin receptors in the basal ganglia, and this may have important implications in the pathogenesis of delayed neurodegeneration caused by enviormental neurotoxicants. (supported by the Parkinson and Movement Disorders Program research fund).

1714 ABSENCE OF SPECIFIC CCK-A BINDING SITES ON HUMAN PANCREATIC MEMBRANES

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Cholecystokinin (CCK) induces satiety in rodents, dogs, and humans, an effect mediated by the CCK-A receptor subtype. CCK analogs may thus be useful drugs for controlling obesity. However, CCK-A agonists induce acute pancreatitis in rodents and dogs, and have been shown to potentiate azaserine-induced preneoplastic changes in rat pancreas, an effect attributed to the trophic action of these compounds. Since administration of CCK to humans has not produced evidence of pancreatic injury, we evaluated human pancreas for the presence or absence of specific CCK-A binding sites, using the CCK-A selective ligand [^{3}H]L-364, 718. The receptor was detected in pancreatic membrane preparations from rat and guinea pig, with binding parameters similar to published values. It was also detected in dog pancreas, a finding that has not to our knowledge been previously reported, but was not present in any of six human pancreata obtained from organ donors. Absence of the receptor in human pancreas was not a result of tissue deterioration, since 1) the muscarinic receptor was detected (using [^{3}H]-N-methylscopolamine) in human pancreas lacking CCK-A binding sites; 2) the CCK-A receptor was readily detected in rat pancreas stored, processed, and frozen under the same conditions as the human tissue; and 3) the receptor was readily detected in gallbladder from a human pancreas donor. Since human pancreas appears to have no detectable CCK-A binding sites compared to species that are susceptible to CCK-A-mediated pancreatic injury and cell proliferation, adverse pancreatic effects observed following CCK administration to rodents and dogs may not be relevant to humans.

1715 INHIBITION OF TUMOR NECROSIS FACTOR α INDUCED-NITRIC OXIDE SYNTHASE BY TYROSINE KINASE INHIBITORS IN RAT HEPATOCYTES

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Tumor necrosis factorα (TNFα) induces nitric oxide synthase (NOS) in rat hepatocytes. However, the mechanism of this induction is unknown. We have used the tyrosine kinase inhibitor genistein to examine the role that protein tyrosine kinases may play in NOS induction. Genistein inhibited TNFα induction of NOS in a dose-dependent manner when added to hepatocyte cultures 15 min prior to the addition of TNFα. NOS induction was measured by media nitrate/nitrite levels (an indirect measure of enzyme activity) and by NOS immunoblotting (indicating protein levels). The ability of genistein to inhibit NOS induction was diminished when added 45 min after TNFα suggesting an early role for tyrosine kinase activity in NOS induction. Herbimycin A, a tyrosine kinase inhibitor which is mechanistically and structurally distinct from genistein, also inhibited TNFα induction of NOS. The data suggest the presence of protein tyrosine kinase activity on the TNFα signal transduction pathway in primary rat hepatocyte cultures. Interestingly, genistein inhibited induction of NOS by xanthine/xanthine oxidase (an oxygen radical generating system), and by PMA (a phorbol ester), implying tyrosine kinase activation is a common process in NOS induction. Furthermore, genistein inhibition of xanthine/xanthine oxidase-induced NOS may signify that the tyrosine kinase activity being examined is regulated by oxidative stress.

1716 A THROMBIN-GENERATED FRAGMENT OF OSTEOPONTIN UPREGULATES MITOGENIC RESPONSIVENESS OF SMOTH MUSCLE CELLS IN A CHEMICAL MODEL OF ATHEROGENESIS

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Recent studies in this laboratory have shown that thrombin cleavage of osteopontin (OPN), an α_v integrin ligand, is associated with the upregulation of mitogenic responsiveness in vascular smooth muscle cells (SMCs) induced by allylamine (AAM). The current studies were designed to examine the ability of AAM challenge *in vitro* to modulate OPN gene expression and to determine if thrombin-cleaved OPN (tOPN) influences the mitogenic behavior of SMCs. Naive rat aortic SMCs were challenged with AAM (1–100 nM) for 4 hr and subcultured once before assessment of OPN gene expression and DNA synthesis. AAM challenge in vitro at 10, but not 1 or 100 nM, increased DNA synthetic profiles and OPN mRNA levels in SMCs. Recombinant OPN synthesized using a reticulocyte transcription/translation system, or tOPN generated *in vitro* by addition of 5 units/ml thrombin, did not influence DNA synthesis in G_0-synchronized naive SMCs. However, tOPN markedly enhanced the mitogenic effect of epidermal growth factor (10 ng/ml), a poor SMC mitogen. These data indicate that a thrombin-generated fragment of OPN is required for the enhanced mitogenic responsiveness of vascular SMCs induced by AAM, and implicate cross-regulation of growth factor-/integrin-mediated signaling in the regulation of SMC growth and differentiation. (Supported by NIH grants ES 04849 and 00213).

1717 BENZO(A)PYRENE INDUCES TRANSCRIPTION OF THE NORMALLY CONSTITUTIVE C-HA-RAS PROTOONCOGENE IN VASCULAR SMOOTH MUSCLE CELLS

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Benzo(a)pyrene (BaP) and other polycyclic aromatic hydrocarbons have been implicated in the initiation and/or progression of atherosclerotic lesions in laboratory animals and in humans. Previous studies in this laboratory have demonstrated that deregulation of protooncogene expression, and in particular c-Ha-ras, is linked to the induction and maintenance of altered vascular smooth muscle cell (SMC) phenotypes by BaP *in vivo* and *in vitro*. The present work examined the ability of BaP to influence c-Ha-ras expression, a protooncogene expressed constitutively in SMCs. Northern analysis demonstrated that c-Ha-ras mRNA levels increase in SMCs treated with 3 μM BaP. This response was mediated by transcriptional activation of the gene as reflected by nuclear run-on experiments. Electrophoretic mobility shift assays showed that BaP induced formation of discrete DNA-binding protein com-

plexes that interact with the c-Ha-ras promoter at sites with extensive homology to the xenobiotic response element (XRE). These experiments suggest that c-Ha-ras gene expression is modulated by BaP at the transcriptional level and raise provocative questions regarding the promotional basis of BaP-induced atherogenesis and carcinogenesis. (Supported in part by NIH grants ES 04849 and ES00213).

1718 THE SENSITVITY OF CYP1B1 - CATALYZED POLYCYCLIC AROMATIC HYDROCARBON METABOLISM TO PROTEIN KINASE C ACTIVITY

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The metabolism of 7,12 - dimethylbenz(a)anthracene in C3H10T1/2 mouse embryo fibroblasts (10T1/2) is catalyzed by cytochrome P4501B1 (CYP 1B1) which is highly induced by polycyclic aromatic hydrocarbons (PAH) and by 2,3,7,8 - tetrachlorodibenzo-p-dioxin (TCDD). A 10T1/2 cell line that stably overexpressed cDNA encoding the β form of protein kinase C (PKCβ) demonstrated a 4 - fold inhibition of basal and PAH -induced cellular and microsomal metabolism as well as CYP 1B1 protein expression. The PKC activator (TPA, 100nM) and inhibitors (staurosporin[St] 0.1pM) and chelerythrine chloride[CC] 0.4μM) augmented these inhibitory effects which also correlated with decreased CYP1B1 mRNA expression. TPA, St. and CC, also almost completely suppressed basal and TCDD - induced catalytic, protein and mRNA expression of CYP1B1 in standard 10T1/2 cells after short (6h) or long (12h) exposures. We have used anti - PKC antibodies to show substantial suppression of PKC protein in cell lysates by TPA and St. These data suggest that PKC is essential for both basal and AhR - induced expression of CYP1B1. Recent studies have suggested that PKC is neccessary for the formation of AhR - ARNT heterodimers. We will report studies of the effects of PKC on complex formation between AhR, ARNT and oligonucleotides containing various CYP1B1 XRE domains using 10T1/2 nuclear extracts.
(Supported by NIH Grant 144 - BY87).

1719 NITROGEN DIOXIDE-INDUCED EXPRESSION OF PLASMA MEMBRANE ANNEXIN II IN PULMONARY ARTERY ENDOTHELIAL CELLS

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Exposure to nitrogen dioxide (NO_2) results in activation of plasma membrane-dependent signal transduction and upregulation of a number of proteins in pulmonary artery endothelial cells (PAEC) (Am. J. Respir. Crit. Care Med. 149:A454, 1994). Because NO_2 exposure alters plasma membrane-dependent functions, we examined whether exposure to NO_2 is responsible for upregulation of plasma membrane proteins in PAEC. Confluent monolayers of PAEC incubated in medium containing [[^{35}S]-methionine were exposed to 5 ppm NO_2 or air (control) in 5% CO_2 for 24 hr at 37°C. After exposure, plasma membranes were isolated and proteins were separated by two-dimensional gel electrophoresis and analyzed by staining with Coomassie blue dye as well as by autoradiography. Exposure to NO_2 resulted in 8/9-fold increase of protein with 36 kD molecular mass compared to control. N-terminal amino acid sequence analysis revealed that the first 20 amino acids of NO_2-induced 36 kD protein had a 100% match with amino acid residues from porcine annexin II (lipocortin II). Immunoblot analysis of NO_2-induced 36 kD protein using monoclonal antibody further confirmed the identity of annexin II. These results demonstrate that exposure to NO_2 results in upregulation of plasma membrane annexin II, an important second messenger. Synthesis of annexin II may be associated with the overall activation of the signal transduction pathways in response to NO_2-induced modulation of metabolic events in PAEC.

1720 A23187 AND AROCLOR 1242 ACTIVATE DISTINCT PLA_2s WHICH ARE DIFFERENTIALLY LINKED TO EICOSANOID PRODUCTION AND SUPEROXIDE ANION GENERATION IN NEUTROPHILS

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Arachidonic acid (AA) released from membrane phospholipids by phospholipase A_2 (PLA_2) is important as a substrate for formation of eicosanoids and as a second messenger for superoxide anion (O_2.) generation in neutrophils. Not all agents which stimulate AA release cause O_2. production, and this may be due to activation of different isoforms of PLA_2. This study was undertaken to test the hypothesis that distinct PLA_2 are activated by Aroclor 1242, which stimulates O_2. production, and A23187, which does not. Both Aroclor 1242 (0.1–10 μg/ml) and A23187 (0.2–20 μM) caused concentration-dependent release of ^{3}H-AA from rat, peritoneal neutrophils; however, significant O_2. production was seen only in response to Aroclor 1242. Greater than 85% of the total AA released in response to A23187 was converted to eicosanoids, while less than 20% was metabolized by neutrophils stimulated with Aroclor 1242. Omission of calcium from the medium abolished release of AA in response to A23187, but did not alter Aroclor 1242-induced AA release. AA release and O_2. production in response to Aroclor 1242 were inhibited in a concentration-dependent manner by haloenol lactone suicide substrate (HELSS), an inhibitor of calcium-independent PLA_2s. HELSS did not significantly alter AA release in response to A23187. These data suggest that the PLA_2 activated by Aroclor 1242 is calcium-independent and liberates AA for stimulation of O_2. production. In contrast, the PLA_2 activated by A23187 is dependent upon calcium and is coupled to eicosanoid production. Supported by NIH grant ESO4911.

1721 THE EFFECTS OF LEAD ACETATE ON RESISTANCE ARTERY FUNCTION IN RATS

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Low level exposures to lead (Pb) in both animals and humans have been associated with hypertension independent of imparied renal function. We hypothesize that low levels of lead impair the normal relaxation response to nitric oxide of the resistance artery vasculature. Female rats were exposed to lead acetate (25 ppm) via drinking water for 90 days. Mesenteric arteries from the exposed animals were examined in an *in vitro* pressurized arteriograph system. Constriction to phenylephrine and relaxation to methacholine (receptor mediated nitric oxide synthase stimulator) and sodium nitroprusside (nitric oxide generator) were compared to control females. Maximal relaxation to sodium nitroprusside (SNP) was reduced in the lead treated animals (89 ± 2.8%) verses controls (97 ± 1.5%)($p < .03$). EC50's to both drugs suggest that the activity of guanylate cyclase is impaired (methacholine 0.01μM for control vs 0.03μM for lead treated ($p < .08$), SNP 0.009μM vs. 0.03μM ($p < .08$)). The reduction in maximal response and sensitivity to SNP indicates that low levels of lead can adversely affect the relaxation capacity in mesenteric resistance arteries by altering the response to nitric oxide. These data suggest a possible mechanism of lead toxicity in the resistance vasculature at the level of the guanylate cyclase instead of nitric oxide synthase, because relaxation to SNP does not require nitric oxide synthase. A decrease in relaxation capacity in resistance arteries could translate into an increased peripheral resistance and/or alterations in vascular reactivity *in vivo*.

1722 THE FUNCTION OF MOTIF II OF THE VOLTAGE-DEPENDENT CALCIUM CHANNEL

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Intracellular Ca^{2+}, which increases mainly through voltage-dependent calcium channels (VDCCs), plays an important role in the function of all excitable cells, including muscle, neuronal and secretory tissues. VDCCs exist as complexes of the main subunit α_1 and the auxiliary subunits α_2/δ, β and γ subunits. The α_1 subunits are encoded by at least six distinct genes and have different pharmacological and biophysical properties. In order to define the structure-function relationship of VDCCs, a chimeric Ca2+ channel α_1 subunit was constructed, in which the motif II of 1,4-dihydropyridine (1, 4-DHP) sensitive rabbit cardiac α_1 subunit is replaced by the corresponding region of the 1, 4-DHP-insensitive rabbit brain α_1 subunit (BI). The chimeric subunit was expressed in *Xenopus oocytes* along with α_2 and β subunits. The resulting Ca2+ channel had similar inactivation kinetics relative to that of the parent BI channel, but it maintained 1,4-DHP sensitivity and had unitary conductance similar to the parent cardiac Ca2+ channel. These data suggest that motif II may contain structural components important for inactivation of the voltage-dependent calcium channel, but does not contribute to the difference in 1,4-DHP sensitivity and channel conductance. (Supported by NIH P01 HL22619-15 (A. S.), 5R37 HL43231-04 (A. S.) and T32 HLO7382-16 (A. S.))

1723 ENDOTHELIAL STRUCTURAL INTEGRITY IS MAINTAINED DURING ENDOTOXIC SHOCK IN A TYPE-1 INTERLEUKIN-1 RECEPTOR KNOCKOUT MOUSE

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The derangement of arterial endothelial cell morphology is a good indicator of a severe shock state. Since interleukin-1 (IL-1) has been implicated in this process, we examined the structural integrity of aortic endothelial cells during endotoxemia in animals genetically devoid of the type 1 IL-1 receptor. Endotoxin (10 mg/kg *Escherichia coli* injected intraperitoneally) (LD_{100}) or saline vehicle was administered to adult male C57BL/129J wild-type control mice and C57BL/129J knockout mice possessing a homozygous deletion of the type-1 IL-1 receptor. The integrity of the aortic endothelium was determined by comparisons of ultrastructure. Mice injected with sterile vehicle showed normal endothelial ultrastructure with intact membranes. Wild type and knockout control animals receiving saline vehicle showed complete maintenance of aortic endothelium (29.11 ± 0.27 and 30.85 ± 0.21 intact endothelial cells/mm of internal elastic lamina (IEL), respectively, p = N.S.) Endotoxin treated wild type animals showed extensive endothelial damage with most sections showing only denuded internal elastic lamina on the luminal surface (1.83 ± 0.38 cells/mm IEL, $p<0.001$ vs control). Knockout animals treated with endotoxin showed complete maintenance of endothelial structural integrity (34.08 ± 0.57 cells/mm IEL, $p<0.001$ vs endotoxin treated wild type) with ultrastructural morphology appearing identical to those given saline vehicle. The maintenance of endothelial integrity in animals devoid of the IL-1 receptor confirms earlier observations of endothelial cell protection with IL-1 receptor antagonism and suggests that IL-1 contributes significantly to sepsis-induced endothelial damage.

1724 ANTIMONY INDUCTION OF HEAT SHOCK PROTEINS IN NEONATAL RAT CARDIAC MYOCYTES

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Antimony is a hard, brittle metal that has extensive uses in industry. Antimony-containing compounds are used in the manufacture of paints, ceramics, pyrotechnics, fire retardants and glass. The National Institute for Occupational Safety and Health estimates that more than 250,000 individuals in the United States are exposed to antimony compounds at work. Antimony-containing compounds produce cardiac functional alterations and toxicity in both experimental animals and humans. Previously, we reported that potassium antimonyl tartrate (PAT) induced oxidative stress and was directly toxic to cultured neonatal rat cardiac myocytes. Little is known, however, regarding the biochemical effects of nonlethal concentrations of PAT on cardiac myocytes. Numerous studies have reported the induction of stress or heat shock proteins in a wide variety of cellular systems following exposure to metals. Heat-shock proteins are a ubiquitous group of proteins that are synthesized in response to heat and other environmental stress and are thought to afford protection against cell toxicity. The stress protein heme oxygenase is especially susceptible to induction by metals. Drummond and Kappas demonstrated that PAT as well as other antimony-containing compounds were potent inducers of heme oxygenase in the liver and kidney of rats, but the effects of PAT on the heart were not examined. In the present study, sublethal concentrations of PAT were examined in neonatal rat cardiac myocytes for PAT-induced changes in cellular glutathione levels, heme oxygenase and stress protein expression. Our results indicate that an 18 hr exposure to low concentrations of PAT (5–25 μM) increases cellular glutathione levels, heat shock protein 70 and heme oxygenase activity in cardiac myocytes in a concentration-dependent manner.

1725 SULFUR MUSTARD INDUCTION OF DERMAL MICROBLISTERS IN A WEANLING PIG MODEL

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Sulfur mustard (SM; 2,2'-dichloroethyl sulfide) can produce incapacitating blisters in humans following dermal exposure. The hairless guinea pig (HGP) is one of the few animal models which has been shown to exibit microscopic epidermal-dermal separation (microblisters) following SM exposure. Because the HGP is in short supply, studies were conducted to confirm that weanling pigs exhibit microblisters and to evaluate the utility of the pig as an alternative model for studying the blistering process. Weanling pigs were percutaneously exposed for varying lengths of time to SM. One day following exposure, skin light reflectance measurements were taken to assess erythema. The animals were sacrificed and skin specimens were taken, processed, and stained for histopathological evaluation. Results from these studies demonstrate that the weanling pig skin exhibits erythema and pathologic changes similar to those reported in SM-exposed HGP skin. These data indicate that the weanling pig could be used as an alternative model to the HGP for studying microblister. [Supported by USAMRDC Contract DAMD17-89-C-9050]

1726 SUBCHRONIC TOXICITY ASSESSMENT OF LY315535, A 5-HT AGONIST/MUSCARINIC ANTAGONIST IN BEAGLE DOGS

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Daily oral doses (0.8, 2 or 5 mg/kg) of LY315535, a potent 5-HT1a agonist with mild muscarinic antagonist activities, were administered to beagle dogs for 1 month. At 5 mg/kg, clinical signs were restricted to tremors, increased muscular tone and hypersalivation. Occasional convulsions, reduced activity and abnormal gait were also observed; These clinical signs were minimal at 2 mg/kg. Tolerance to central nervous system effects did not develop. The drug-related clinical signs were of limited duration and were likely to be the result of the pharmacological mechanism of action of LY315535. Transient decreases in body weight gain were observed in animals receiving 2 or 5 mg/kg and increases in respiratory rates were observed in animals given 5 mg/kg (5-fold), and 2 mg/kg (2-to 3-fold). Heart rate was significantly increased in dogs given 5 mg/kg. In a single female from this group (5 mg/kg), sinus tachycardia, increased PQ duration, increased alanine aminotransferase (2-fold), aspartate aminotransferase (7-fold) and creatine phosphokinase (27-fold) activities were noted. Except for these findings, the only notable change in clinical chemistry was an increase in cholesterol values in dogs given 5 mg/kg. No changes in serum gastrin levels and morphometry of the stomach wall were detected. Medial hemorrhagic necrosis was observed in the small arteries of the heart, gastrointestinal tract, kidney and thymus of a single high-dose male dog. This type of arterial lesion has been reported with vasoactive drugs (Van Vleet *et al.*, 1991). The lesions observed in this study may be due to the action of LY315535 on arterial smooth muscle 5-HT receptors (Mylecharane, 1990).

1727 ETHANOL POTENTIATES THE DEPRESSANT EFFECTS OF COCAINE IN HUMAN FETAL MYOCARDIUM IN VITRO

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Cocaine and ethanol use is wide spread in our society and is not uncommon in pregnant women. Previous work from this center demonstrated that acute exposure to moderate concentrations of cocaine or ethanol, singly, produced significant effects on fetal myocardial action potentials and contractility *in vitro* and may provide a mechanism to account for fetal arrhythmias or sudden death *in utero*. The use of fetal tissue in this *in vitro* study was approved by the University of South Florida's Institutional Review Board which governs policies and procedures for research involving human subjects. Fetal hearts (12-14 weeks) were obtained at the time by elective abortion and transported to the laboratory in cold physiological salt solution (PSS). In the laboratory, a small portion of the ventricular wall (~2 × 6 mm) was pinned, endothelial surface up, in a specially constructed Sylgard-coated 2ml plexiglass perfusion chamber. The force of muscle contractions and transmembrane potentials were measured simultaneously. Developed force was measured with a microforce transducer, and transmembrane potentials using standard 3M KCl-filled glass microelectrodes. Preparations were stimulated at 2 Hz with supramaximal electrical pulses which were delivered across bipolar platinum electrodes. Throughout the course of an experiment fresh PSS was oxygenated and superfused through the tissue bath. The temperature in the bath was maintained at 37°C. When a concentration of cocaine that singly produced moderate myocardial depression (200ng/ml) was administered to fetal myocardium equilibrated with PSS containing ethanol at a final concentration of 0.02%, significant depression and or block of action potentials and contractility was observed. Ethanol (0.02%) singly did not produce any significant effects on the heart. This study suggests that the combined use of cocaine and ethanol may produce myocardial depression greater than that predicted from the effects of these chemicals when used individually.

1728 ELECTROCARDIOGRAPHIC EFFECTS OF CYANIDE ON THE ISOLATED, PERFUSED RAT HEART

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We have been studying the cardiotoxicity of emetine for several years. In the course of mechanistic studies exploiting cyanide's effect on glycolysis, we had the opportunity to record electrocardiograms from isolated, perfused rat hearts during perfusion with either 1 mM or 2 mM sodium cyanide. Simultaneously, contractions were recorded through a needle placed into the left ventricle and release of lactate dehydrogenase into the coronary effluent was measured. Cyanide perfusion had a profound slowing effect on the heart rate, it had little effect on AV-conduction as measured by the PR-interval, and it decreased the duration of ventricular depolarization as measured by the QRS-duration. As previously reported, cyanide provided some protection against the toxic effects of emetine in our model. We have ascribed this effect of cyanide to stimulation of glycolysis.

1729 MEASUREMENT OF CARDIOVASCULAR RESPONSE OF MALE SPRAGUE DAWLEY RATS TO MODULAR ARTILLERY CHARGE SYSTEM USING RADIOTELEMETRIC IMPLANTS

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The U.S. Army is developing an artillery propellant which is a granular mixture of 98% nitrocellulose, nitroglycerin, nitroguanidine, and 2% cryolite and graphite. The propellant charge is contained within a rigid, combustible case. As part of the process to develop health effect criteria, a series of studies was performed. Because of the presence of nitrates, it was necessary to evaluate the cardiovascular effects of repeated exposure. Blood pressure and heart rates of Sprague-Dawley rats containing radiotelemetric implants were monitored 24 hours per day for 7 days. Baseline evaluations were performed 7 days prior to and 7 days following treatment. The propellant was ground to a powder and mixed with powdered feed. Animals were allowed to eat *ad libitum*. Dosage levels in the feed were 0.0, 0.2, 1.0, and 2.0 g/kg of propellant. A treatment-related increase in diastolic blood pressure and heart rate (107 and 105% respectively) was noted. These differences were significant at $p < 0.01$. The increase in diastolic pressure suggests a reduction in cardiac output, necessitating an increase in heart rate. (Supported by DOD Contract No. F33615-90-C-0532).

1730 DIRECT ACTION OF THE δ ISOMER OF HEXACHLORO-CYCLOHEXANE ON VENTRICULAR CARDIOMYOCYTES AND RYANODINE RECEPTOR FUNCTION

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Delta-hexachlorocyclohexane (δ-HCH) has been shown to modulate ryanodine-sensitive Ca^{2+} channels of sarcoplasmic reticulum (SR) and contractility of rat atrial strips (Pessah, *et. al., J. Pharmacol. Exp. Therap.* **262:** 661-9, 1992). Recently δ-HCH was shown to mobilize Ca^{2+} from an IP_3sensitive stores and inhibit depletion-activated Ca^{2+} entry in rat basophilic leukemia cells (Mohr, *et. al., Molec. Pharmacol.*, In press). In the present study, the cardiotoxic actions of δ-HCH are investigated further in single ventricular myoctes by measuring changes in the rate of cell shortening (video edge motion detection) and intracellular Ca^{2+} transients (using the dye indo-1) in electrically stimulated single cardiomyocytes isolated from guinea pig. Single cardiac SR Ca^{2+} channels reconstituted in the planar lipid bilayer under voltage clamp were used to demonstrate and characterize the direct actions of δ-HCH. δ-HCH (10μM) inhibits cell shortening and force of contraction by ~60%. δ-HCH (10μM) reduced the peak amplitude and increased duration of the Ca^{2+} transients by 28% and 20%, respectively. These effects of δ-HCH on the cardiomyocytes were dose-dependent. Measurements of gating kinetics of single cardiac Ca^{2+} release channels in the planar lipid bilayer show that δ-HCH dramatically increases open probability and mean open time without altering unitary channel conductance. The actions of δ-HCH at the cellular and single channel levels appear to be isoform-selective since γ-HCH (lindane) is significantly less potent in inducing these effects. These results confirm a novel mechanism by which δ-HCH alters Ca^{2+} signaling in the heart. Supported by a pilot project from the Environmental Health Sciences Center (NIH ES05707).

1731 CARDIOVASCULAR PROFILE IN THE YUCATAN MICROPIG-ANESTHETIZED AND CONSCIOUS-RESTRAINED AND UNRESTRAINED MODELS

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In instances where species suitability becomes an issue (ex. low bio-availability or atypical sensitivity etc.), the Yucatan micropig can be considered as an alternative to dogs or primates for determining the effects of a compound on the cardiovascular system. Under isoflurane anesthesia, pigs were instrumented with specialized catheters for measurements of pulmonary artery pressure, systemic arterial pressure, left ventricular pressure, heart rate, cardiac output, ECG and body temperature. Cardiovascular profiles were monitored in anesthetized pigs (n = 12), conscious pigs restrained in a sling (n = 18) and conscious, unrestrained pigs using a jacket and tether system (n = 3). All animals received placebo intravenous injections or infusions of saline. Some animals also received intravenous injections of Diphenhydramine HCl. A consistent response was observed between models, however, the data were most stable in the anesthetized model, followed by the conscious unrestrained model and conscious restrained models, respectively. Although an increased incidence of artifact (including cardiac arrhythmias) was observed when compared with dogs or monkeys, it was concluded that the Yucatan micropig can be successfully used as an alternative to the dog or monkey for anesthetized and conscious restrained/unrestrained cardiovascular profiling. Given the potential for reactions with anesthetic and relatively stable baseline, the conscious unrestrained model will in many cases be the model of choice, however, the potential cardiac sensitivity of the pig should be taken into account when considering any of these models.

1732 ELEVATION OF HAPTOGLOBIN LEVELS IN THE SKIN OF WEANLING PIGS FOLLOWING PERCUTANEOUS EXPOSURE TO SULFUR MUSTARD

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Sulfur mustard (SM; 2,2'-dichloroethylsulfide)-induced skin injury is characterized by erythema, edema, basal keratinocyte toxicity, and epidermal-dermal separation (microblisters). Current endpoints used to assess skin damage caused by SM include measurements of edema and erythema, and histopathology. Studies were performed to examine SM-induced alterations of proteins in weanling pig skin to identify possible biomarkers that could be used to quantitatively assess irritation. Weanling pigs were exposed percutaneously to SM and sacrificed 24 hr following exposure. Full-thickness skin samples were taken from the lesion site, from immediately adjacent to the lesion site, and from a control site approximately 1 inch from the lesion. The samples were homogenized in cold buffer containing protease inhibitors and the supernatant from centrifugation were subjected to one-dimensional polyacrylamide gel electrophoresis. Changes in relative protein molecular weight profiles of Coomassie Blue stained gels were assessed by image analysis. Gels not stained with Coomassie Blue were used for western blot analysis of haptoglobin, an acute phase reactive protein. Haptoglobin levels were elevated in lesion site samples compared to skin from control sites. The haptoglobin acute phase response may be an indicator of severity of SM-induced inflammation and may prove useful as a quantitative measure of SM-induced skin injury.
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1733 PRECLINICAL TOXICITY ASSESSMENT OF TNK-tPA IN DOGS FOLLOWING IV BOLUS ADMINISTRATION

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The toxicity of single and multiple doses of TNK-tPA, a recombinant tissue plasminogen activator variant, was assessed in beagle dogs. In an acute study, 2 dogs/sex/group received a single IV bolus (0.3–30 mg/kg) dose of TNK-tPA followed by a challenge dose on Day 14. Coadministration with aspirin (ASA) and heparin was also evaluated. In a multi-dose study, 4–6 dogs/sex/group received a daily IV bolus (0.3–10 mg/kg) dose of TNK-tPA for at least 8 days, or 90-min. IV infusion of Activase®(rt-PA) for 14 days. In both studies, the presence of arginine in the vehicles was associated with angioedema. TNK-tPA was antigenic after a single dose; dogs given a challenge dose (30 mg/kg) showed severe signs of anaphylaxis followed by death. Similar signs of anaphylaxis were observed in the multi-dose study, beginning on Day 8 of dosing. This is not an unexpected response following administra-

tion of a heterologous protein. TNK-tPA produced exaggerated pharmacological effects on blood indices: prolonged bleeding times, bleeding from previous venipuncture sites, and changes in clinical pathology parameters. Coadministration of ASA and heparin did not potentiate the effect of TNK-tPA on indices of blood coagulation, cause any additional toxicity nor affect the disposition of TNK-tPA. Comparable effects with regards to vehicle toxicity, antigenicity and blood indices were seen with 10 mg/kg of rt-PA. Since TNK-tPA is a protein similar to rt-PA, which is used extensively in humans with no vehicle toxicity and minimal or no antigenicity, these effects are likely not a concern in clinical trials with TNK-tPA.

1734 ARRHYTHMOGENESIS FOLLOWING ADMINISTRATION OF THE QUINOLONE PD138312 TO DOGS

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PD 138312, 7-[3-(1-amino-1-methylethyl)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro- 1,4-dihydro-4-oxo-1, 8-naphthyridine-3-carboxylic acid, hydrochloride, a broad spectrum quinolone antibacterial agent, was administered orally to beagle dogs at 50, 100, and 200 mg/kg for 2 weeks. PD 138312 was clinically well tolerated at 50 mg/kg. Repeated doses of 100 and 200 mg/kg resulted in emesis, decreases in food consumption, accompanied by body weight loss and moribundity. Electrocardiographic changes including prolongation of the QT (and QTc) interval were noted for all treated animals. Holter monitor records showed ventricular ectopic beats, including bigeminal ventricular ectopic beats in all treated groups of both sexes during Week 2 of study. The total number of ventricular ectopic beats was variable (ranging from 1 to approximately 47,000 over 24 hours), showing no dose relationship, and occurred predominantly from approximately 2 through 10 hours postdose. No treatment-related histopathologic changes were noted in the heart. The profile of toxicity seen with PD 138312, including the cardioarrhythmogenic changes, has been previously reported in dogs given quinolones. Since these arrhythmias occurred without associated pathological changes in the heart, PD 38312 may interfere directly with electrocardiophysiologic impulses.

1735 EFFECT OF ACUTE COCAINE EXPOSURE ON CELL VIABILITY EVALUATED IN AN ISCHEMIA/ REPERFUSION MODEL OF PRIMARY CULTURES OF RAT MYOCARDIAL CELLS

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Cardiovascular complications associated with cocaine abuse have been well documented. The mechanisms of cocaine-induced cardiotoxicity have not been clearly elucidated. Cocaine has been suggested to be directly toxic to myocardial cells. However, recent studies have shown that acute cocaine exposure did not significantly decrease cell viability in cultured cardiomyocytes under normoxic conditions. Because cocaine is capable of producing myocardial ischemia *in vivo*, the present study was undertaken to evaluate the role of ischemia in cocaine-induced cytotoxicity in primary cultures of rat myocardial cells. Cells were incubated with cocaine with or without the presence of norepinephrine for 3 and 6 hr under normoxic conditions. There was no obvious cytotoxicity observed in normoxic cells treated with cocaine alone or cocaine plus norepinephrine. In an ischemia/reperfusion model, cytotoxicity was not evident after 90 min of ischemia. But, during 60 and 120 min of reperfusion, ischemic cells were significantly injured when compared to non-ischemic cells and to cells kept in a low pH buffer. The presence of cocaine and norepinephrine during the ischemia/reperfusion period did not enhance the observed injury with ischemia/ reperfusion alone. Our results suggest that ischemia may not potentiate cocaine toxicity. (Supported by NIH grant DA07918–01A2).

1736 THE EFFECTS OF INHIBITING SEMICARBAZIDE-SENSITIVE AMINE OXIDASE ACTIVITY ON EXTRACELLULAR MATRIX

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Semicarbazide-Sensitive Amine Oxidases (SSAO) are ubiquitous enzymes found in many animal and plant tissues. Although the physiological function(s) of SSAO remain unclear, activity is particularly high in vascular smooth muscle. Previous research generated interest into a possible role for SSAO in extracellular matrix (ECM) production and/or maintenance. Aortas of weanling rats treated with SSAO inhibitors showed histopathological alterations of collagen and elastin with resultant pathophysiology. Therefore, to further examine the relationship between SSAO and ECM, neonatal rat vascular smooth muscle cells (VSMC) were treated *in vitro* with selective enzyme inhibitors: SSAO dose-dependent inhibition was confirmed using semicarbazide (SEMI) and MDL 72, 145A (MDL / Marion Merrell Dow). Cell growth was unaffected by treatments. Because ascorbic acid (Vit. C) is crucial to normal ECM production, the relationship between Vit. C and SSAO activity was characterized. In agreement with previous research, total ECM was increased with addition of exogenous Vit. C (by 30% in this study). Vit. C greatly increased SSAO activity; however, concomitant addition of SEMI negated this increase. Interestingly, cotreatment (Vit. C plus SEMI) resulted in the same ECM profile of collagen/ elastin as Vit. C alone. Inhibition with SEMI alone significantly lowered SSAO activity and reduced elastin by 70%. With MDL inhibition, however, collagen decreased (53%) and elastin drastically increased (118%). The elastin fraction appeared grossly abnormal and is undergoing further study. The collagen and elastin changes highlighted in this study demonstrate a role for SSAO in the functional and visual alternations of ECM observed in previous *in vivo* studies. This work was supported by: T32ES07254 and HL-26189.

1737 SINGLE AND MULTIPLE DOSE PHARMACODYNAMIC STUDIES OF TWO METHEMOGLOBIN-FORMING COMPOUNDS

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Current therapy for cyanide intoxication consists of inducing methemoglobinemia with sodium nitrite and providing sulfane sulfur with sodium thiosulfate. Oral (gelatin capsule) single and multiple dose experiments were conducted to determine dose-MHb concentration curves for two MHb-forming compounds. Single dose experiments used a cross-over design. In multiple dose experiments, six dogs were given a loading dose of 2.0 mg WR242511/kg with subsequent dosings of 1 mg/kg every 48 hr, or 6 mg WR269410/kg every 8 hr. Blood samples were collected for hemoglobin and MHb analyses. For WR242511 single dose experiments, peak MHb levels (mean of 7.84%) were greater than 5% with minimal variability, were reached approximately 3 to 4 days after dosing, and MHb levels returned to approximately 5% within 5 to 8 days. In WR242511 multiple dose experiments, most animals reached 95 percent of steady state MHb (mean of 12%) during the fourth dose period (6 to 8 days after initial dose) and remained near that level until the experiment was terminated. For WR269410 single dose experiments, peak MHb levels (mean of 7.29%) were highly variable with 2 of 6 animals below the targeted 5% MHb level. In WR269410 multiple dose experiments, most animals reached 95 percent of steady state MHb (mean of 9.42%) during the seventh dose period (range of 2 to 20 days). Peak MHb levels greater than 30% were observed in five animals and resulted in early termination of the study. [Supported by USAMRDC DAMD17-89-C-9050]

1738 CARDIOVASCULAR AND RENAL RESPONSE OF ANF84 AND URODILATIN IN FEMALE RHESUS MONKEYS

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ANF84 is a synthetic atrial natriuretic peptide (ANP) analog with the following features: (1) it is a selective agonist for receptor guanylyl cyclase-A, (2) it does not bind the clearance receptor, and (3) it is more resistant to degradation by neutral endopeptidase (NEP). Urodilatin, a natriuretic factor from kidneys, has an amino acid sequence identical to that of ANP, although extended at the amino terminus by four residues. This molecule has the same receptor selectivity as ANP but, like ANF84, is resistant to NEP degradation relative to ANP. The objective of this study was to compare ANF84 and urodilatin in two alert, chair-restrained female rhesus monkeys (random cross-over design with 2-week washout). Vehicle alone (5% dextrose) was infused for 60 minutes (2 ml/kg/hr) followed by a 2-hour infusion of ANF84 or urodilatin at a rate of 10 ng/kg/min (2 ml/kg/hr). The primary variables for comparison were central arterial blood pressure, heart rate, urine flow, and sodium, potassium, and cGMP excretion. Urine flow increased significantly with ANF84; the effect was more subtle with urodilatin. Correlation plots were used to examine the difference between the effects of ANF84 and

urodilatin. For ANF84, there was a linear correlation between the urine sodium and flow and a nonlinear (power function) correlation between urine potassium and flow. A multivalued relationship existed between cGMP and urine flow such that the hysteresis loop demonstrated an advance rather than a delay of the cGMP as compared with the urine flow. With urodilatin, there was no relationship between sodium, potassium and cGMP, and urine flow at this dose. Minor depressor effects on mean arterial pressure and heart rate were observed with ANF84 but not with urodilatin.

1739 EFFECTS OF FUMONISINS ON CARDIOVASCULAR FUNCTION IN SWINE

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Fumonisins (FB), mycotoxins produced by *Fusarium moniliforme*, induce liver damage and, at lethal doses, acute pulmonary edema in swine. We examined the cardiovascular responses to FB in male cross-bred pigs fed fumonisin-containing culture material at 0 or 20 mg/kg/day (FB_1 and FB_2) in a nutritionally balanced diet for 7 days. On day 8, pigs were anesthetized and surgically catheterized. Mean pulmonary artery pressure, central venous pressure, systemic arterial pressure, left ventricular end-diastolic pressure, heart rate and cardiac output were measured, and stroke volume and *dP/dt* max values were calculated. Upon recovery from anesthesia, these measurements were repeated, and arterial and mixed venous blood gas samples were evaluated. Pigs fed fumonisin had marked decreases in *dp/dt max*, systemic arterial pressure, heart rate, and cardiac output accompanied by significant increases in left ventricular end-diastolic pressure and mean pulmonary artery pressure. Treated pigs had significant decreases in both arterial and venous blood O_2 tension. These results suggest that overall cardiac function of pigs is compromised by fumonisin treatment. This study also provides evidence suggesting that the development of fumonisin-induced pulmonary edema in swine is cardiogenic in origin, possibly resulting from acute left-sided heart failure.

1740 CELL-SPECIFIC METABOLISM IN BENZENE TOXICITY. A METABOLIC BASIS FOR BENZENE-INDUCED TOXICITY AT THE LEVEL OF THE PROGENITOR CELL IN HUMAN BONE MARROW

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A potential mechanism underlying the myeloid toxicity of benzene is bioactivation of its phenolic metabolites by peroxidases. Peroxidases are present in high concentration in bone marrow in the form of myeloperoxidase (MPO). Benzene is converted to phenol and further metabolized to hydroquinone by cytochrome P4502E1 (2E1). MPO catalyzes the generation of reactive quinones from phenol, catechol and hydroquinone and these quinones can be detoxified by NAD(P)H: quinone oxidoreductase (NQO1). We have examined the levels of MPO, NQO1 and 2E1 in critical subpopulations of human bone marrow - stroma and progenitor cells (CD34+, >96% pure). MPO and NQO1 in human stromal cultures varied as a function of time with MPO decreasing and NQO1 increasing. MPO but not NQO1 could be detected in human bone marrow progenitor cells using activity assays and western blots. 2E1 could not be detected in either stroma or progenitor cells using western blots. This data shows that CD34+ have marked MPO content and a high MPO/NQO1 or activation/detoxification ratio. Progenitor cells, which are generally regarded as the initial target cells for the development of leukemias, were previously thought of as MPO-negative. This data provides a metabolic basis for benzene-induced toxicity at the level of the progenitor cell in human bone marrow (Supported by ES 04112 and the American Petroleum Institute).

1741 THE IN VIVO HEMATOTOXICITY OF 1,2,4-BENZENETRIOL (BT) TO FEMALE BALB/C MICE

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Previous work in our laboratory demonstrated that the in vivo administration of the polyhydroxylated benzene metabolite, BT, to Balb/c mice increased nitric oxide production in the bone marrow. BT also produced oxidative modifications and inhibited granulocytic differentiation in human promyelocytic leukemia cells (HL-60), a cellular model system. In order to elucidate the role of BT in benzene toxicity, the current experiments focused on characterizing its in vivo hematotoxicity. Female Balb/c mice were treated intraperitoneally with PBS vehicle or 25 mg/kg BT in PBS for 4 consecutive days. Animals were sacrificed 24 hours following the last treatment, peripheral blood, spleen and bone marrow were collected and cell suspensions of nucleated cells were prepared. Cells were enumerated and differential counts were obtained by flow cytometry. In comparison to controls BT treated animals had increased spleen to body weight ratios (114 % of control) and decreased hematocrit (87 % of control). The most significant effect of BT was to depress the number of lymphocytes in the bone marrow (55 to 61 % of control) in the absence of a significant effect on the total number of nucleated cells in the bone marrow. The total number of peripheral blood cells as well as the number of peripheral blood lymphocytes was also decreased by BT treatment (64 % and 55 % of control respectively). Preliminary experiments suggest that this effect is not observed in animals treated with HQ. These data suggest that lymphocytes are a specific target of BT toxicity.

1742 THE TOXICOLOGIC EFFECTS OF CI-1010, A NITROIMIDAZOLE RADIOSENSITIZER, IN BEAGLE DOGS

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CI-1010, (R)-α-[[(2-bromoethyl)amino]methyl]-2-nitro-1H-imidazole-1-ethanol monohydrobromide, is an hypoxic cell radiosensitizing anticancer agent. Four dogs/sex/ group were dosed intravenously once per day, 5 days/ week, for 3 weeks at 0, 1, 3, 7 (females) or 10 (males) mg/kg, followed by an 8 week recovery period. A histaminergic reaction with erythema, skin swelling, urticaria, and pruritus occurred imediately post-dosing at all doses. Hypotension occurred as well following administration of 30 or 40 mg/kg in a separate study. Deaths associated with myelosuppression occurred at 7 and 10 mg/kg. Hypoactivity, hyperthermia, pallor, weakness, red-tinged feces, and petechiae were seen in moribund animals at these doses. Leukopenia and thrombocytopenia were minimal at 1 mg/kg (≤43%, with all counts within historical range), but profound at 7 or 10 mg/kg (≥82%; ≥94% in moribund animals). Statistically significant decreases in bone marrow total nucleated cell counts occurred at 3, 7, and 10 mg/kg, and in moribund animals marrow smears showed a virtual absence of proliferating myeloid and erythroid cells. Histologically, bone marrow hypocellularity at ≥3 mg/kg, and seminiferous tubule degeneration at all doses, were observed. All histopathologic changes were reversible except those in the testes, in which lesion severity progressed during the recovery period.

1743 CROSSMATCHING BLOOD TYPES FROM FOUR RAT STRAINS

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The ability to collect multiple blood samples from a single animal without disrupting the pharmacodynamics of the animal or endangering its life is of concern when conduction pharmacokinetic or metabolism studies. However, if an equal amount of blood could be transfused to the animal after each collection, it would reduce the likelihood of altered pharmacodynamics or endangerment to life. The objective of this research was to determine if blood from one animal could be transfused to another and if there are incompatibilities between strains. The study examined four rat strains (Fischer, Sprague-Dawley, Wistar and Long Evans). Each strain consisted of four males. Four milliliters of whole blood were collected from each animal for crossmatching. Blood samples were first cross-matched with each animal within its strain and then with each animal of the other three strains. The blood samples were then pooled by strain and the pooled samples crossmatched against each other. Finally, all four strains were pooled together and crossmatched. No incompatibilities were found within each of the strains or between them, whether individual or pooled.

1744 OXIDATIVE STRESS IN SPLENOTOXICITY OF ANILINE

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Aniline-induced splenic toxicity is characterized by hemorrhage, capsular hyperplasia, fibrosis and a variety of sarcomas in rats. Early biochemical events in the spleen which could lead to the observed effects are not known. Our preliminary studies using single and multiple (4 and 7) doses of 1 mmole/

kg of aniline hydrochloride(AH) in rats demonstrated that AH could induce lipid peroxidation and protein oxidation in the spleen and significant increases were observed at 4 doses. Therefore, a dose-response study of AH has been performed. Male Sprague-Dawley rats were given 4 doses each (1 dose/day) of 0.25, 0.5, 1 and 2 mmole/kg of AH in tap water by gavage, while controls received tap water only. Animals were euthanized 24 hr following the last dose and tissues obtained. Spleen weight increased by 32 and 80% at 1 and 2 mmole/kg doses, respectively. Splenic lipid peroxidation showed dose-dependent increases of 24, 32 and 43% at 0.5, 1 and 2 mmole/kg, respectively. Protein oxidation in the spleen, quantitated as carbonyl content per mg protein, showed 10, 28 and 27% increases at 0.5, 1 and 2 mmole/kg, respectively. Iron content in the spleen also showed dose-dependent increases of 72, 172 and 325% at 0.5, 1 and 2 mmole/kg, respectively. Dose-related histopathologic expansion of splenic red pulp was characterized by increasing vascular congestion (most pronounced at 2 mmole/kg) and increased red pulp cellularity and cellular fragmentation at 1 and 2 mmole/kg; iron deposition in red pulp also increased dramatically with dose. No changes were observed at 0.25 mmole/kg dose. These studies establish that aniline induces lipid peroxidation and protein oxidation in the spleen in a dose-dependent manner and indicate the role of oxidative stress in the splenic toxicity of aniline.

1745 THE MOUSE ERYTHROBLAST ASSAY (MEA): A NOVEL SCREEN FOR THE SUPPRESSION OF ERYTHROPOIESIS

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An *in vitro* model system using mouse erythroblasts was examined for the ability to rapidly screen xenobiotics for potential suppression of erythropoiesis. Swiss-Webster mice (25 – 35 grams) received phenylhydrazine (60 mg/kg/day for 2 days) with splenic erythroblasts being harvested 72 hr after the last treatment. Erythroblasts were cultured for 24 hr in 96 well Millipore multiscreen filtration plates with erythropoietin and test compounds. [^{3}H]Thymidine incorporation was determined following a two hour incubation. A number of compounds were examined in this assay including specific and non-specific growth modulators (erythropoietin, insulin, cholera toxin, TPA, conconavalin A, and EGF), agents known to inhibit erythropoiesis (etoposide and methotrexate) as well as two immunosuppressants which do not affect erythropoiesis (cyclosporine A and FK-506). Erythropoietin, TPA and cholera toxin produced concentration-dependent increases in [^{3}H]thymidine incorporation. However, insulin, conconavalin A and EGF had no effect indicating that erythroblasts were the predominant responding cell type. Etoposide and methotrexate produced concentration dependent decreases in [^{3}H]thymidine incorporation with EC_{50} values of 1.5 and 402 ng/ml, respectively. Cyclosporine A and FK-506 only affected [^{3}H]thymidine incorporation at cytotoxic concentrations (i.e., >25 μg/ml). Acetaminophen (which has been reported to produce toxic effects in the bone marrow of mice) had an EC_{50} of 231 μg/ml although this effect was greatly reduced by the inclusion of catalase and superoxide dismutase into the culture media. In summary, these data indicate that the MEA will require additional validation but it shows promise as a screen for detecting compounds that suppress erythropoiesis.

1746 EVALUATION OF THE TOXIC EFFECTS OF DAPSONE AND ITS DERIVATIVES

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Dapsone (DDS) is used as a treatment of leprosy. Recently, DDS has shown an anticonvulsive effect, expanding its therapeutic spectrum. Sustained DDS doses produce hemolytic anemia, through the action of an active metabolite, N-hydroxyl-DDS. In the present study, we evaluated the toxic effects of two dapsone derivatives with anticonvulsive action and their amino groups blocked: acedapsone (ADDS) and glucosulfone (GDDS). To assess toxicity we measured the following parameters: Hemoglobine (Hb), erythrocytes count (EC) and reduced glutathione content (GSH) in blood of rats treated with drugs for 1 month, using the respective median effective doses against convulsions. DDS decreased Hb (26%) EC (27%) and GSH (94%), while ADDS diminished 10.9%, 8.4% and 93% the same parameters. GDDS decreased HB (4.4%), EC (9.1%) and GSH (8.8%). GDDS seems to be the best option for clinical trials as antiepileptic, due to its low hematic toxicity.

1747 PRECLINICAL SAFETY EVALUATION OF RECOMBINANT HUMAN THROMBOPOIETIN (rhTPO)

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rhTPO is a hematopoietic growth factor being developed as a potential therapeutic for the treatment of thrombocytopenia. To support the safe use of rhTPO in humans, the acute and multi-dose (4-week) toxicity of rhTPO was evaluated in mice and monkeys. In mice, single intravenous (IV) or subcutaneous (SC) doses of up to 5000 μg/kg were generally well tolerated. A dose of 5000 μg/kg (IV or SC) resulted in effects which included slight/moderate clinical pathological changes and megakaryocytic hyperplasia. In multi-dose studies, findings in IV treated mice included: 1) inflammatory lesions at the IV injection site (tail), 2) mild/marked clinical pathological changes (generally attributable to tail lesions), and 3) increases in absolute and relative heart, lung, spleen and liver weights. Microscopic findings noted in the bone marrow at the 4-week sacrifice included megakaryocytopoiesis (doses ≥ 50 (IV) and 250 μg/kg/day (SC)) and a decrease and increase in the proliferating erythroid and myeloid pools, respectively (IV doses ≥ 50 μg/kg/day). In monkeys, single IV/SC doses produced a dose-related increase in platelets (doses ≥ 100 μg/kg) and/or bone marrow megakaryocytic hyperplasia (5000 μg/kg). Similar effects upon megakaryocytes and platelets and mild/moderate reductions in RBC counts were observed following multiple dosing (IV/SC doses ≥ 10 μg/kg/day). Together, these data warrant further investigation of rhTPO as a treatment for thrombocytopenia and support the hypothesis that rhTPO is a relatively selective stimulator of platelet production.

1748 MYELOTOXICITY OF 5-FLUOROURACIL TO THE HEMATOPOIETIC PROGENITORS FROM MICE -IN VIVO-IN VITRO CORRELATION

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Myelotoxicity of 5-fluorouracil(5-FU), which is one of the antimetabolic anicancer drugs, was investigated *in vitro* and *in vivo* using mouse bone marrow cells. Results : *In vitro* ; Reduction in the number of granulocyte-macrophage colony formation unit(CFU-GM) by 5-FU with continuous exposure for 7 days ($IC_{50} = 9.69 \times 10^2$nM) was greater than that following 1 hour exposure ($IC_{50} = 1.06 \times 10^5$nM). Erythroid progenitor cells were more sensitive to the toxicity of 5-FU following 1 hour exposure than myeloid progenitor cells when comparing the IC_{50} values of CFU-GM. *In vivo*; The kinetic study of CFU-GM colony formation was performed in mice after a single i.v. injection of 5-FU at concentrations of 25–150mg/kg. The time which showed the most severe toxicity to myeloid progenitor cells after the injection of 5-FU was dose dependently delayed and the severity of toxicity to myeloid progenitor cells was correlated with the doses of 5-FU. Conclusion: There was a good correlation between the *in vitro* concentrations and the *in vivo* doses of 5-FU when compared with the same degree of CFU-GM colony formation. We will confirm these results by using the pharmacokinetic data in mice.

1749 ROLE OF LIPID PEROXIDATION IN DAPSONE-INDUCED HEMOLYTIC ANEMIA

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Dapsone hydroxylamine (DDS-NOH) is a direct-acting hemolytic agent responsible for dapsone-induced hemolytic anemia in the rat. Hemolytic activity is associated with the appearance of disulfide-linked hemoglobin adducts on certain membrane skeletal proteins, and we have previously postulated that this lesion initiates removal of damaged red cells from the circulation by splenic macrophages. However, an alternate hypothesis is that removal of DDS-NOH-damaged red cells is the result of membrane lipid peroxidation. To test this alternate hypothesis, rat and human red cells were incubated with hemolytic concentrations of DDS-NOH (125–250 μM), and examined for evidence of lipid peroxidation using malondialdehyde (MDA) formation and paranaric acid degradation. The extent of MDA formation and paranaric acid degradation in DDS-NOH-treated rat and human red cells was not significantly different from control cells. In contrast, MDA formation and paranaric acid degradation was significantly increased in rat and human red cells treated with hemolytic concentrations of phenylhydrazine (0.5–5 mM). These data indicate that lipid peroxidation is not involved in the mechanism

underlying dapsone-induced hemolytic anemia. Furthermore, the data suggest that dapsone and phenylhydrazine induce a hemolytic response by different mechanisms. Supported by NIH grant HL-30038.

1750 AN INVESTIGATION OF HEALTH CONCERNS ATTRIBUTED TO REFORMULATED GASOLINE USE, SOUTHEASTERN WISCONSIN

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Under the Clean Air Act Amendments of 1990, reformulated gasoline (RFG) containing a minimum of 2% oxygen by weight was to be exclusively sold in ozone nonattainment areas, including the Milwaukee and Chicago metropolitan areas, by Jan. 1, 1995. Between January and March, 1995, government agencies received 3515 telephone calls from Milwaukee residents concerned about health effects related to RFG use, while few such calls were received from Chicago residents. In response, Wisconsin government agencies conducted an investigation consisting of a random telephone survey of 500 residents of each of three areas: the Milwaukee metropolitan area, the Chicago metropolitan area, and Wisconsin outside the Milwaukee metropolitan area. Univariate and multiple logistic regression models were used to determine predictors of 15 "unexplained" health symptoms. Predictors analyzed in the models included demographic characteristics, health status, exposure to specific RFG components and awareness of RFG issues. Significantly more Milwaukee residents (23%) than Chicago (6%) or Wisconsin (6%) residents reported "unusual" symptoms, and the strongest predictors of symptoms in logistic regression models ($p<0.01$) were having suffered a cold or flu since Nov. 1, 1994, cigarette smoking and awareness of RFG issues, while exposure to specific RFG components was not predictive of symptoms. The same survey was also administered to individuals telephoning government agencies with health concerns ("health contacts") who identified themselves. Analysis of this group indicated that 33% of those calling had called at least once previously. Among the 1339 "health contacts" who were located and agreed to participate, older, retired individuals and those with allergies were disproportionately represented, and no specific symptom syndrome was evident. The explanation for the observed pattern of health contacts is unclear from this data: either older, retired individuals and those with allergies are more sensitive to the health effects of RFG exposure or they are more aware of the agencies which will accept their calls. The results of this study do not support the conclusion that RFG use is associated with widespread or serious acute adverse health effects in the general population.

1751 ASSOCIATION BETWEEN ASTHMA SYMPTOMS AND AIR POLLUTANT EXPOSURE, CENTRAL WISCONSIN

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Asthma prevalence was determined among children in a central Wisconsin county using an internationally standardized symptom survey. Exposure to ambient air pollutants in the county, which contains four paper mills in the southeastern portion, was estimated at each child's school and residence with an air pollutant dispersion model which included information on permitted emissions of sulfur dioxide (SO2) and estimated yearly emissions of SO2, hydrogen sulfide (H2S) and methanol. The model of permitted emissions predicted peak three-hour exposure (SO2 range 0–500 ug/m3) while the models of estimated yearly emissions predicted average annual exposures (SO2 range 0–5 ug/m3). The survey was mailed to the parents of first and second grade children and administered to seventh and eighth grade children at school during 1993–1994. All county public school districts except one and four private schools participated. Responses were received from 1229 (60%) of the younger children and 1824 (82%) of the older children. "Doctor-diagnosed" asthma was reported by 9% of the younger children and 11% of the older children (Wisconsin fraction, 8% ± 3%) and was consistent in all school districts except one located in an area of high SO2 emissions. Parental asthma and childhood respiratory diseases were predictive of asthma symptoms in both age groups, while parental cigarette smoking or pet ownership were not. Accuracy of the model was assessed using measurements at a SO2 monitor located in the county and the correlation of self-perceived odors with modelled H2S exposures for each child. Among the older children, asthma medication use associated with modelled 3-h peak SO2 exposure (odds ratio 1.12 per 100 ug/m3 of 3-h peak SO2 exposure; $p<0.05$) but were not associated with exposure to the other pollutants modelled or to annual estimated SO2 concentrations. The results of this study are a preliminary indication that results of pollutant dispersion models might be used to predict ambient concentrations at individual locations and that estimates of 3-h yearly maximum SO2 exposure at a child's home and residence may be associated with asthma symptoms.

1752 IMMUNOTOXICITY OF MIXTURES OF POLYCHLORINATED AROMATIC HYDROCARBONS WITH METHYLMERCURY AT ST. LAWRENCE RIVER FISH LEVELS ON FISCHER 344 RAT LYMPHOCYTES IN VITRO

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Methylmercury (MHg), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCCDs) and dibenzofurans (PCDFs) are immunotoxic environmental pollutants. However, toxicity of their mixtures at levels found in fish in St. Lawrence River is unknown. Based on Environmental Québec fish levels analysis report, rat splenocytes, thymocytes and peripheral blood cells (PBC) were exposed *in vitro* to MHg (0.1, 2 μg/ml), PCDD/PCDF mixtures (1, 15 pg/ml) of 3 PCDDs (2,3,7,8-tetraCDD, 1,2,3,7,8-pentaCDD and 1,2,3,4,7,8-hexaCDD) and 2 PCDFs (2,3,7,8-tetraCDF and 1,2,3,7,8-pentaCDF) or 3 Aroclors; 1242, 1254 and 1260 PCB mixtures (0.01, 0.5 μg/ml) or the possible combinations of the MHg/PCB/PCDD/PCDF mixtures. DNA synthesis in cells stimulated with concanavalin A (Con A), phytohemagglutinin (PHA) or lipopolysaccharide/dextran sulphate was determined by ^{3}H-thymidine uptake, cytotoxicity and intracellular Ca^{++} by flow cytometer. Cytotoxicity in splenocytes occurred at 4–72 hr with 2 μg/ml MHg and mixtures with 2 μg/ml of MHg. Intracellular Ca^{++} levels were unaffected. MHg and all MHg/PCB/PCDD/PCDF mixtures suppressed proliferation of splenocytes, thymocytes and PBC to the mitogens. PCB or PCDD/PCDF mixtures enhanced proliferation of splenocytes and PBC to Con A and PHA by 20–30%. No interactions (additive or antagonistic) were observed with the MHg/PCB/PCDD/PCDF mixtures. Thus of the St. Lawrence River contaminants fish levels, MHg represents a potential risk to the immune system of fish-eating animals/mammals.
Supported by TOXEN and CNTC.

1753 IN VITRO IMMUNOTOXICITY IN RATS OF MIXTURES OF POLYCYCLIC CHLORINATED AROMATIC HYDROCARBONS AND MERCURY CONTAMINANTS FOUND IN FISH FROM THE SAINT-LAURENT RIVER

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Several chemicals contaminating fish such as polychlorinated dibenzo dioxins (PCDDs), polychlorinated dibenzo furans (PCDFs), polychlorinated biphenyls (PCBs) and mercury depress the immune system at various degrees, however, very few data exists on the immunotoxicity potential of mixtures of these chemicals. Based on concentrations measured by Environnement Québec in the flesh of fish from the Saint-Laurent river, mixtures containing various congeners were prepared: 3 PCDDs, 2 PCDFs, 19 PCBs and methyl mercury (Hg). These mixtures were prepared using all possible combinations of two concentrations of the following three groups of chemicals: 1 and 15 pg/mL for the PCDDs/PCDFs group, 10 and 500 ng/mL for the PCBs, and 0.1 and 2.0 μg/mL for Hg. Isolated splenocytes and peripheral blood neutrophils (PBN) from male Sprague-Dawley rats (300 – 400 g) were exposed *in vitro* to the chemical mixtures. Cytotoxicity toward splenocytes was observed after 4, 24 and 48 h of exposure to mixtures containing the highest Hg concentration (2.0 μg/mL). A similar pattern of effects was observed when oxidative burst of PBN was considered. Our data indicate that at environmental concentrations, of all the contaminants considered in this study, Hg presents the greater risk to the immune system. Furthermore, no interaction between the chemicals forming the mixtures could be detected.
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1754 CRITERIA FOR IMMUNOLOGICAL DATA FOR ANALYSIS BY MULTIVARIATE METHODS

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The quantitative relationship between immune parameters and host resistance to cancer or infections disease is not well understood. Elucidation of such relationships would provide information relevant to risk assessment of envi-

ronmental exposures. Multivariate statistical methods can be effective in quantitating these relationships, however, the limitations of such methods must be considered. These considerations were explored using data from B6C3F1 female mice administered dexamethasone (0.3-30.0 mg/kg/ day) subcutaneously for 16 days. A host resistance model using B16F10 melanoma cells was used and immune parameters were measured including spleen and thymus weight, macrophage function, CD4/8 subpopulations in thymus and spleen, B220/Ia subpopulations in peritoneum, NK cell activity, and peripheral blood differentials. For multivariate analysis, two independent experiments of 40 mice with 8 mice per group were used. Additionally, two other experiments each of 40 mice measured resistance to B16F10 tumors. A large number of observations is required for multivariate methods. Thus, by combining the two experiments, one data set of 80 observations was produced. To demonstrate that incorporation of unique experiments into one data set was a viable method, values obtained within one treatment were randomized. Thus simulates the merger of experiments where the matching of immune parameter values from mice within a single treatment is unknown. Using multivariate methods, this set was compared to the original data set with the correctly matched observations for each mouse. Principal components analysis (PCA) was performed on the immune parameters. Subsequent multiple regression analysis was done using the factor (PCA) as the independent variables with tumors resistance as the dependent variable. Reproducible models between the original and the randomized data set were obtained when certain criteria were met. Variation within a treatment of an experiment must be limited. This can be determined by the coefficient of variation which generally must be less than 30%. Thus, experiments must be done using standard conditions to achieve low variability within treatments. Developing criteria for incorporation of several experiments into a large data set appropriate for analysis by multivariate methods can provide more insight into the relationships between immune parameters and host resistance.

1755 CLINICAL IMMUNOLOGIC EFFECTS OF LEAD EXPOSURE IN WORKERS EMPLOYED AT A SECONDARY SMELTER

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NIOSH, in collaboration with NIEHS, studied a comprehensive panel of clinical immunological outcomes in workers employed at a secondary lead smelter and in workers at a hardware manufacturing company without occupational lead exposure (unexposed). This study is by far the largest to date investigating the immunologic effects of lead among workers for workplaces that operate within the OSHA lead standard. All study participants were male. 145 exposed and 84 unexposed workers participated. Endpoints evaluated included: hematology, lymphocyte immunophenotyping, clinical chemistries including sera immuoglobulin levels, lymphocyte proliferation and natural killer cell cytolysis. The median blood lead level (exposed) was 39 μg/dl (range: 15–55 μg/dl) and <2 μg/dl (range: <2–12 μg/dl) among the unexposed group. The median zinc protoporyhrin (ZPP) was 48 μg/dl (range: 2–424 μg/dl) among the exposed and 17.5 μg/dl (range: 1–59 μg/dl) among the unexposed group. Analyses of raw data indicated statistically significant changes in selected parameters (when compared to the unexposed group) including changes in serum IgG and IgA levels, salivary IgA, percent of CD3+ cells and CD19+ cells. Analyses of covariates (age, race, smoking, alcohol use, etc.) is ongoing; however, the relationship between the number of CD3+/CD4+ T-cells and exposure varied by age. Among workers under 30 years of age, there was no difference in the number of CD3+/CD4+ T-cells between the unexposed and exposed group. But among workers 30 years of age or older, exposed workers, on average, had fewer CD3+/CD4+ T-cells. These results emphasize the importance of covariate analyses in human clinical immunological studies.

1756 ORAL EXPOSURE TO 2-BUTOXYETHANOL ALTERS IMMUNE RESPONSES IN BALB/c MICE

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Several members of the glycol ether family have been reported to induce immunotoxic effects in rodent models. We report here the effects of oral exposure to 2-butoxyethanol (BE) on immune function in BALB/c mice. Female mice were dosed by oral gavage at 50, 150 or 400 mg/kg body weight for 10 consecutive days followed by a 3-day rest. No effects were observed at any of the BE concentrations on body weight, spleen and thymus cellularity or spleen:body weight ratio and thymus:body weight ratio. There was a 3-fold increase in concanavalin A mitogenic stimulation of splenocytes from mice at all BE concentrations tested compared to control. Lipopolysaccharide stimulation was significantly elevated only in splenocytes from mice at the 400 mg/kg BE dose. The mixed lymphocyte reaction was increased over control by 2 fold at all BE concentrations. Cytotoxic T lymphocyte activity was significantly increased in both the 150 and 400 mg/kg dose groups compared to vehicle-exposed mice. This study demonstrates that BE is an immunomodulating agent and that the mouse model responds differently than previously reported results in the rat. This research was supported in part by NIH AREA grant # ESO6782-01A1.

1757 MORPHOLOGIC LESIONS AND ACUTE IMMUNOTOXIC EFFECTS IN TILAPIA (Oreochromis niloticus) EXPOSED TO BENZO[a]PYRENE

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Tilapia were exposed by intraperitoneal injection to the polycyclic aromatic hydrocarbon (PAH), benzo[a]pyrene (B[a]P). Histopathologic evaluation of the fish primary hematopoietic compartment, the pronephros, demonstrated increased vacuolation of both stromal and parenchymal cells, as well as dramatic reduction of lymphoid elements. Total pronephros cell counts were also diminished in a dose-dependent manner by the chemical exposure. The oxidative metabolic burst in phorbal myristate acetate (PMA)-stimulated macrophages isolated from the pronephros was significantly inhibited by B[a]P, but only at the highest dose level employed. The phagocytic capacity of pronephros macrophages was not altered by the chemical treatment. In contrast, the proliferative response of splenic T and B lymphocytes to mitogens Con A and LPS, respectively, was significantly decreased by B[a]P. These data correspond well with similar immune alterations in laboratory rodents exposed to B[a]P, indicating fish may respond similarly to mammals following PAH contamination. Further, tilapia exposed to B[a]P at doses not producing overt toxicity showed immune alterations consistent with impaired immunity in mammals, and thus may be in a state of clinically-significant immunosuppression.

1758 MODULATION OF NK1.1 SPLENOCYTES AFTER EXPOSURE TO OCTAMETHYLCYCLOTETRA-SILOXANE (D4)

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Octamethylcyclotetrasiloxane (D4) is a low molecular weight, cyclic polydimethylsiloxane which is present in many consumer goods including over-the-counter medications, personal care and household products. It has previously been shown that natural killer (NK) cell activity is augmented in B6C3F1 mice following exposure to D4. When mice were gavaged daily with 1000 mg/kg for 35 days, significant enhancement was apparent by day 5, peaked on day 7, then returned towards baseline by day 10. Spleen weight and cell counts tended to increase whereas splenic cellularity decreased following exposure for 7 days. Histopathology of the spleen revealed increased hematopoiesis characterized by confluent red pulp foci consisting primarily of erythroid precursor-like cells. Cell population analysis by flow cytometric methods indicated that the percentage of $NK1.1^+/CD3^-$NK cells and $NK1.1^+/CD3^+$ T cells were not significantly altered after D4 exposure although the total number of NK cells per spleen tended to increase. $NK1.1^+/CD3^+$ T cells have been shown to exhibit NK like cytolytic activity against YAC cells. Results of T cell depletion studies in conjunction with population studies suggest that the increased cytolytic activity is a function of enhanced activity of NK $1.1^+/CD3^-$ NK cell on a per cell basis. This augmentation may be due to increased binding of the NK cell to its target, increased killing capabilities or increased recycling. The effect may be mediated via direct or indirect mechanisms. Supported by NIEHS-T32-ES07087.

1759 EFFECTS OF CAPSAICIN ON THE IN VITRO ANTIBODY RESPONSE OF HUMAN AND RODENT LYMPHOCYTES

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Capsaicin (CAP: 8-methyl-N-vanillyl-6-nonenamide) is a major pungent

component of hot peppers. In this study, effects that CAP and its metabolites, vanillin(VN), vanillic acid(VA), vanillyl alcohol(VAL), and 4-hydroxy-3-methoxybenzylamine(HMB) have on an in vitro polyclonal antibody response(PAR) by mouse, rat and human lymphocytes were studied. Mouse, rat and human lymphocytes were activated by lipopolysaccharide, *Salmonella typhimurium* mitogen, and heat-inactivated *Staphylococcus aureus* respectively and IgM produced in culture supernatant was measured by ELISA. For all of the species studied. CAP and its metabolites suppressed PAR of lymphocytes in a dose-dependent manner. The potential suppressive effects were as following: Mouse: HMB > VAL > VAC > CAP ≥ VN Rat: HMB > VN > VAC ≥ VAL > CAP Human: CAP –ill– VAC ≥ HMB ≥ VN > VAL 100 μM of CAP produced 78.2% suppression of PAR in human lymphocytes while it produced 15.4 and 27.6% suppression of those in mouse and rat lymphocytes respectively. 100 μM HMB produced 46.3, 91.2 and 31.4% suppression of PAR in mouse, rat and human lymphocytes respectively. These results indicate that there are species differences in CAP and its metabolites-induced suppression of PAR by human and rodent lymphocytes.

1760 THE CYTOSKELETON AS POSSIBLE TARGET FOR THE THYMUS ATROPHY INDUCING COMPOUND DBTC

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The organotin compound di-n-butyltin dichloride (DBTC) is well-known to induce thymus atrophy. Flowcytometry has demonstrated that DBTC inhibits the proliferation-dependent differentiation of immature CD4-CD8-into CD4+CD8+ thymocytes.
To understand the mechanism underlying the selective action of DBTC we have studied the effect of DBTC on three distinguishable processes that are regarded important in thymocyte differentiation, i.e. interaction between thymocytes and thymic epithelial cells (TEC), TcRgagb-CD3 signalling and apoptosis. It was found that DBTC inhibits the binding of thymocytes to TEC and also the capping of TcRgagb-CD3 and of CD8. However, DBTC increases the release of Ca^{2+} elicited by triggering the TcRgagb-CD3 complex without inducing a Ca^{2+}-influx by itself. Together with the finding that cytochalasin B also caused an increase of TcRgagb-CD3 signalling and the observation that microvilli are present at the site of binding between thymocytes and TEC we suggest that the dithiol reagent DBTC specifically affects cytoskeletal functioning. We also found that DBTC induces DNA fragmentation after a 4h culture period when cells were shortly (10 min) preincubated. A direct effect of DBTC on the cytoskeleton might also underly the induction of apoptosis. The relation of the in vitro effects to the mechanism of organotin-induced thymus atrophy is unknown as yet.

1761 IMMUNOTOXIC EFFECTS OF A COMBINATION OF TRIMETHOPRIM: SULFAMETHOXAZOLE AND ZIDOVUDINE (AZT) IN BALB/c MICE

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Trimethoprim: Sulfamethoxazole (T:S, 5:1) is used in combination with AZT to treat opportunistic infections in AIDS patients. We evaluated the potential of T:S alone or in combination with AZT, in a regimen similar to that expected in the treatment of an AIDS patient, to alter hematological and immunological parameters in female BALB/c mice when given once daily by oral gavage for 28 days. Neither T:S at a dose of 1500, nor AZT at 240 mg/kg showed any effect on any of the parameters measured. Of 6 mice treated with the combination of T:S at 1500 and AZT at 240 mg/kg, 2 died prior to termination of the study. The remaining mice showed statistically significant decreases in WBC, RBC, platelet and reticulocyte counts in peripheral blood. Significant decreases in Con A-and LPS-induced T and B cell proliferation, and in percentages of splenic macrophages and B cells, were observed relative to untreated mice or mice treated with either T:S or with AZT alone at the same doses. Proliferation in the MLR was significantly decreased in 2 of 4 mice treated with the combination of T:S and AZT, although cell-mediated cytotoxicity was not affected. The ability of B cells to produce IgM and IgG in responses to KLH immunization was also not significantly different from that of control mice or mice treated with the agents alone. The data suggest that treatment with the combination of T:S and AZT has significant hematotoxic and immunosuppressive effects in female BALB/c mice. (Supported by NIAID-DAIDS contract NO1-AI-15111.)

1762 2',3'-DIDEOXYINOSINE INHIBITS THE SPLENIC AFC RESPONSE IN B6C3F1 MICE TREATED FOR 14, 28 OR 180 DAYS: THE B LYMPHOCYTE IS A PRIMARY TARGET

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2',3'-Dideoxyinosine (ddI) is used for the treatment of HIV positive individuals. The studies reported here were undertaken to determine the time course and the cellular target of the immunotoxicological actions of ddI. B6C3F1 mice were dosed with 100, 250, 500, or 1000 mg/kg ddI in two daily divided doses by oral gavage for treatment periods of 14, 28 and 180 days. The parameters measured were organ weights, body weights, AFC response to sheep red blood cells (sRBC), and serum IgM titers. There was no difference from the control in the organ or body weights except for a dose dependent decrease in spleen weights after 180 days and a 40% decrease in body weights of mice treated with the high dose of ddI for this time period. The AFC response was significantly decreased at all time points by the highest dose of ddI, and by all other doses at the 28 and 180 day dosing periods. Treatment with ddI for 14 days did not decrease the AFC response at doses less than 1000 mg/kg. The serum IgM titers were significantly decreased by all doses after 180 days of treatment, by all but the lowest dose after 28 days, and by only the highest dose after 14 days. A Separation-Reconstitution experiment of the adherent and non-adherent cell populations was performed on the mice dosed for 28 days (1000 mg/kg/day). This revealed a deficit in the ability of both populations to form a normal antibody response to sRBC. A further separation of the T-and B-cells revealed a decrease in the ability of ddI treated B-cells to develop specific humoral immunity. These results suggest that the NOAEL of ddI for treatment periods of 14, 28 and 180 days is 1000 mg/kg, < 100 mg/kg, and < 100 mg/kg, respectively, and that a primary cellular target of ddI is the B lymphocyte. Supported by NIH contract ES 9522.

1763 LOCAL SWELLING AFTER IMPLANTATION OF BIODEGRADABLE POLY-L-LACTIDE IS CAUSED BY TWO DIFFERENT MECHANISMS

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Biodegradable poly(L-lactide) (PLLA) induced in patients a local swelling after three years of implantation. To study the cause of the swelling of PLLA implants and the behaviour of a newly developed poly-DL-lactide (96%L, 4%D-lactide) (PLA96) long-term implantation studies are required. In vitro pre-degradation at elevated temperatures can be used to simulate long term physiological degradation. PLLA, PLA96 and polyethylene (PE) discs were predegraded at 90° C in phosphate buffer of pH 7.4 for 168 h ($PLLA_{168}$, $PLA96_{168}$ and PE_{168}), or 336 h ($PLLA_{LLA336}$). Six samples of pre-degraded and non-degraded discs were subcutaneously implanted per rat. At 2, 4, 8, 12, 26 and 52 weeks postoperatively, animals were sacrificed and the implants were widely excised, fixed and subsequently prepared for histology. Based on in vitro physical analysis, the degradation rate of PLA96 was higher when compared to PLLA. The histological reaction to non-degraded PLLA and PLA96 discs was very mild. The histological reaction to the pre-degraded implants was qualitatively similar to the reaction to the non-degraded implants, however, quantitatively an increase was noted. Histologically, for pre-degraded $PLLA_{336}$ and $PLA96_{168}$ discs an increase of volume was found which was caused by the formation of polymer debris accompanied by a granulomatous inflammatory reaction. Macrophages contained small and large polymer particles, which were found not only in phagosomes, but also in phagolysosomes. However, particle disintegration did not seem to continue within these phagolysosomes. Our results show that the level of degradation obtained in the predegradation process is probably of more importance than the predegradation time. By varying the predegradation time the simulated implantation period can be increased thereby decreasing the risk of missing essential stages in the polymer degradation and accompanying tissue response.

1764 PEPTIDIC KAPPA OPIOID RECEPTOR AGONISTS LACK SIGNIFICANT IN VITRO IMMUNOMODULATORY POTENTIAL

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We have previously demonstrated that in vitro exposure to opioid peptides results in a variety of immunomodulatory sequelae. For example, compounds

acting as μ-opioid receptor agonists exhibit a significant suppression of various immune function parameters. δ-opioid agonists are generally immunostimulatory, whereas δ-opioid antagonists are immunosuppressive. To date there has been only limited information available on the immunomodulatory potential of k-opioid specific compounds. The purpose of this study was to assess the immunomodulatory potential of various peptidic k-opioid receptor agonists using a series of in vitro cellular immune function assays. Naive murine B6C3F1 splenocytes were cultured with Dynorphin A (1–9, 1–11, 1–13 or 1–17) or Dynorphin B at concentrations between 0.00001 and 10 μM. B-cell proliferation was quantitated following cellular activation, T-cell function was assessed by cytokine production, natural immunity was assessed by quantitating natural killer (NK) cell activity following a 24-h exposure in the presence of recombinant interleukin-2 (IL-2), and macrophage function was assessed by IL-6 production. Despite isolated instances of statistical significance, in vitro exposure to any of the k agonists examined in this study was not associated with a consistent pattern of immunomodulation as assessed by B-cell proliferation, T-helper cell cytokine production, macrophage or NK cell function. In all cases, exposure to k-opioid agonists had no significant effect on cellular viability. These data suggest that k-opioid agonists lack significant immunomodulatory potential. (Supported by contract No. 271–91–9201 and grant No. DA-08867 from the National Institute on Drug Abuse).

1765 AMELIORATION OF BMS-191352-INDUCED VASCULAR LEAK SYNDROME IN RATS

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BMS-191352 (BR96 sFv-PE40) is a single-chain immunotoxin fusion protein composed of the BR96 antibody variable regions and a truncated form of *Pseudomonas* exotoxin A that is being developed as an anticancer agent. In rats, BMS-191352 has been shown to produce a dose limiting vasculopathy or "vascular leak syndrome" (VLS). In an attempt to prevent BMS-191352-induced VLS, groups of five male rats were pretreated with either dexamethasone, a corticosteroid; dextran sulfate, a volume expander; ketoprofen, a cyclooxgenase inhibitor; or cobra venom factor, a complement depleter, prior to a single intravenous dose of 12 mg/m^2 BMS-191352.

No deaths occurred and no evidence of VLS (hydrothorax) was noted at necropsy in rats pretreated with 1 mg/kg dexamethasone prior to a single intravenous injection of 12 mg/m^2 BMS-191352.

All rats treated with 12 mg/m^2 BMS-191352 either alone or following pretreatment with dextran sulfate, ketoprofen, or cobra venom factor were found dead 3–4 days after treatment. Deaths were preceded by decreases in serum albumin, globulin and total protein, and by hemoconcentration and an increase in neutrophils. Grossly, hydrothorax 2(often >5 mls), indicative of VLS, was observed in the rats that died.

In conclusion, of the agents tested, only pretreatment with dexamethasone ameliorated BMS-191352-induced vasculopathy in rats.

1766 PRECLINICAL ANTIGENICITY STUDIES OF THE IMMUNOTOXIN BR96-sFv-PE40 (BMS-191352)

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BR96-sFv-PE40 (BMS-191352) is a recombinant DNA-derived fusion protein, composed of the heavy- and light-variable region domains of the monoclonal antibody BR96 and the translocation and catalytic domains of *Pseudomonas* exotoxin A, and is being developed as an anticancer agent. Preclinical safety studies have been performed in rats and dogs, and as part of these studies the antigenicity of the molecule has been assessed. The formation of antibodies was assessed using BR96-sFv-PE40 as a capture reagent in an ELISA assay. BR96-sFv-PE40 is immunogenic following a single dose in both rats and dogs, with positive antibody responses detected as early as Days 7–10. In repeat-dose studies (q3dx5 and q2dx5), the formation of anti-BR96-sFv-PE40 antibodies was associated with rapid declines in plasma levels of BR96-sFv-PE40. In addition, hypersensitivity reactions were observed in those animals that had relatively high titers of anti-BR96-sFv-PE40 antibodies. The hypersensitivity reactions following repeat doses of BR96-sFv-PE40 were associated with rapid declines in functional serum complement (CH50 assay), suggesting that the hypersensitivity reactions were caused by the formation of immune complexes followed by intravascular activation of complement. Treatment with dexamethasone did not prevent the formation of anti-BR96-sFv-PE40 antibodies. However, additional studies are being planned to investigate ways to ameliorate the antigenicity of this potential therapeutic compound.

1767 A SELENIUM-ENRICHED DIET ALTERS THE THYMIC ATROPHY INDUCED BY ORAL EXPOSURE TO T-2 TOXIN IN BALB/c MICE

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T-2 toxin has been previously reported to induce thymic atrophy in both prenatal and adult mouse models. Selenium, an essential micronutrient has been reported to protect against the acute toxic effects of T-2 toxin in rodents. In the present study, female BALB/c mice were fed a defined diet containing either 0.75, 1.5 or 2.5 ppm selenium for 4 weeks. After oral subchronic exposure to T-2 toxin, several immunotoxic endpoints were determined. Total thymus cellularity was reduced to 16% of control and thymus:body weight ratios were decreased to 39% of control by T-2 toxin exposure. All selenium diet groups exposed to T-2 toxin showed an increased cellularity compared with T-2-only treatment. Spleen cellularity was slightly decreased by T-2 toxin treatment but spleen:body weight ratios showed no effect. Selenium dietary exposure had no effect on spleen cellularity or spleen:body weight ratios. Mitogen stimulation with lipopolysaccharide was not affected by T-2 exposure or selenium dietary exposure. Selenium at the lowest dose (0.75 ppm) significantly elevated the concanavalin A mitogenic response but no other effects were observed by selenium or T-2 toxin. Neither the mixed lymphocyte reaction nor the cytotoxic T lymphocyte activity was affected by T-2 toxin or selenium. Supported in part by Northeast Louisiana University Faculty grant funds.

1768 ACUTE IMMUNOTOXIC EFFECTS OF HEXACHLOROCYCLOHEXANE (LINDANE), 7,12-DIMETHYLBENZANTHRACENE (DMBA), AND 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) ON IMMUNE CELL NUMBER AND MACROPHAGE ACTIVITY IN SPLEEN AND PRONEPHROS OF TILAPIA (*Oerochromis niloticus*)

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Tilapia were exposed by intraperitoneal injection to the environmental contaminants lindane, DMBA, and TCDD in separate experiments. Spleen and pronephros cellularities were reduced in a dose-related manner by each chemical agent. The functional capacity of phagocytic cells isolated from both fish spleen and pronephros was also evaluated as an indicator of chemical-induced immunotoxicity. The ability of pronephros and spleen macrophages to phagocytize foreign particles (fluorescent microspheres) was not altered by exposure to lindane, DMBA, or TCDD at levels that did not produce overt toxicity. Phagocytosis was found to be reduced at doses of DMBA associated with reduced activity and increased pigmentation in fish. Similar results were obtained when the macrophage chemiluminescent response was evaluated, with no depression of superoxide radical (hydrogen peroxide) production occurring after exposure to lindane, DMBA, or TCDD. Depression of superoxide radical production occurred at toxic levels of DMBA. These data suggest that total immune cell counts in fish hematopoietic organs may represent more sensitive indicators of environmental immunotoxicant exposure, than does phagocytic activity or chemiluminescent response of macrophages contained within these organs.

1769 IMMUNOSUPPRESSION INDUCED BY CARCINOGEN NNK IN MICE

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NNK (4-methylnitrosamino-1-3-pyridyl-1-butanone) is the most potent carcinogen present in tobacco smoke. It has been shown to induce lung tumors in laboratory animals. The present study was undertaken to study whether it has any effects on the immune system. Female mice of A/J, C57B1/6 or B6C3F1 strain were injected intraperitoneally with different doses of NNK ranging from 50 to 250 mg/kg. One week later the animals were immunized intratracheally with sheep red blood cells (SRBC) and assayed for antibody

responses by the plaque forming cell (PFC) assay. The results indicated a significant inhibition of anti-SRBC PFC responses in the hilar lymph node cells of NNK treated mice compared to the control group. This inhibition of antibody responses was found partly due to the decreased IL-2 secretion by the lymph node cells of NNK treated mice as determined by ELISA. In addition there was severe thymic atrophy as a result of the depletion of $CD4^+CD8^+$ cortical thymocytes in carcinogen treated mice. When tested for antitumor immune responses there was a two fold inhibition of natural killer cell activity in the spleen cells of NNK treated mice which was found to be restored by the injection of rIL-12. These results are useful in understanding why cigarette smokers are more susceptible to respiratory tract infections and lung cancer. This work is supported by NIEHS grant RO1 ES 06363

1770 COMPARISON OF DEXAMETHASONE AND PENTOXIFYLLINE IMMUNOMODULATORY EFFECTS IN THE MYCOBACTERIUM AVIUM-INFECTED MOUSE MODEL

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HIV-infected patients with *Mycobacterium avium* complex (MAC) disease demonstrate significant increases in serum tumor necrosis factor α (TNFα) levels, which is thought to be an important factor in the severe wasting associated with the disease. Drugs which inhibit TNFα production are being sought in an attempt to ameliorate this wasting syndrome. C57BL6 mice which are heterozygous for the *beige* mutation (*bg/+*) are susceptible to infection with *Mycobacterium avium* and demonstrate substantially increased serum levels of TNFα. Dexamethasone (up to 20 mg/kg i.p. given every other day for 3 to 5 weeks) produced a dose-related suppression of serum TNFα levels in MAC-infected C57BL6 (*bg/+*) mice. Dexamethasone also produced an increase in tissue *Mycobacterium avium* burden. Pentoxifylline (up to 30 mg/kg/day i.p. for 3 to 5 weeks) produced only marginal decreases in serum TNFα levels, but no significant increases in tissue mycobacteria were observed. These results indicate that TNFα suppression could increase the severity of MAC disease.

1771 IMMUNOTOXICITY OBSERVED IN MICE EXPOSED TO LOW DOSE OF FORMALDEHYDE

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Formaldehyde (HCHO) is recognized as one of important indoor air pollutants. It is said that HCHO is carcinogen and allergen and has potency to accelerate allergic symptoms in human. We evaluated the effects of HCHO on several immunological parameters. Female B6C3F1 mice were exposed to 100-1800 ppb HCHO daily 8 hr, 6 days in week for 6 weeks. In vitro and in vivo PFC responses against SRBC were enhanced at 200 and 800 ppb HCHO, whereas suppressed in higher doses. Proliferative responses of splenic lymphocytes stimulated by mitogens were also modulated similarly to PFC responses. NK cell activity of splenocytes was enhanced moderately at 100-1000 ppb HCHO. There was no difference of Th/Ts cell ratio of splenic lymphocytes between control and each HCHO exposure groups. Antigen specific IgE production was decreased in 100-1000 ppb HCHO at dose dependent manner. HCHO exposed mice showed early peak of ear swelling response induced by secondary challenge following oxazolon sensitization compare control group. Cytokine mRNA expressions in the macrophages could explain partly the mechanisms of immunomodulations. Our data suggest that HCHO modulates immune responses including allergic responses in broad spectrum. As the results it may cause health disorders observed in HCHO exposed people.

1772 A MECHANISTIC STUDY OF CADMIUM-INDUCED NEPHROTOXICITY: INTERACTION BETWEEN CADMIUM, KIDNEY PROTEINS AND THE IMMUNE SYSTEM

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Cadmium (Cd) is a known nephrotoxin and antilaminin antibodies are increased in the sera of workers chronically exposed to Cd. We speculate that nephrotoxic metals bind to native glomerular proteins (such as laminin or collagen IV) and thereby alter their immunogenicities. This, in turn, leads to an autoantibody response against the native proteins resulting in glomerular damage. To measure the kinetics of the effects of cadmium on antibody and cytokine production, C57BL/6J male mice were exposed to 100ppm $CdCl_2$ in their drinking water for 10, 14 and 28 days. Lymphoproliferation was significantly increased at all time points. In addition, IL-6 levels were significantly elevated at Day 10 and Day 28; while, IL-2 levels were increased at Day 14. In LPS stimulated splenic-cell supernatants from these Cd-exposed mice, IgM and IgA autoantibodies to collagen IV were significantly elevated at Day 10 and Day 28. Furthermore, in antibody acid elution studies of kidney homogenates, total immunoglobulin to laminin, collagen IV and glomerular basement membrane was significantly increased. This data supports the hypothesis that cadmium exposure can activate the immune system to self proteins. Supported by Grant EPA U-914752-01-0.

1773 LIMITED IMMUNOSUPPRESSION RESULTING FROM IN VITRO EXPOSURE TO ACETYLMETHADOLS

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Studies in our laboratory and others have demonstrated that μ-opioid receptor agonists significantly suppress various immune parameters. The purpose of the present studies was to assess potential immunomodulation by acetylmethadols, which are μ-opioid agonists developed for the treatment of drug abuse. This was accomplished by using a panel of in vitro cellular immune function assays. Naive murine B6C3F1 splenocytes were cultured with (±)-α-acetylmethadol, (-/-)-α-acetylmethadol, (±)-β-acetylmethadol or (-/-)-β-acetylmethadol at in vitro concentrations between 0.0001 and 100 μM. B-cell proliferation was quantitated following cellular activation, T-cell function was assessed by cytokine production and natural immunity was assessed by quantitating natural killer (NK) cell activity. All four compounds inhibited B-cell proliferation in a dose-related manner at 10 and 100 μM, although no effect was noted at any other concentration. Likewise, production of IL-2 and IL-4 by T cells was significantly suppressed only at 10 and 100 μM. Cytolytic activity of NK cells was significantly suppressed at all concentrations of either isomer of alpha-acetylmethadol, with greatest suppression observed at 100 μM. Conversely, NK cell function was suppressed only at 100 μM. Of particular note is that immunosuppression was not accompanied by any detectable alteration in cellular viability, suggesting a functional modulation rather than nonspecific toxicity. These data demonstrate that acetylmethadols exhibit immunosuppression only at non-pharmacological concentrations. (Supported by contract No. 271-91-9201 and grant No. DA-08867 from the National Institute on Drug Abuse).

1774 MURINE FOOD ALLERGY TO ALBUMIN DISPLAYS CIRCADIAN RHYTHM

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The importance of establishing a reproducible animal model for human food allergy is increasing among toxicologists. Knowing that there is a marked circadian aspect to allergy, studies were conducted to establish optimum conditions for a mouse model using chicken egg albumin (CEA) as a prototype antigen. Briefly, 5–6 wk old BALB/c were housed 4/cage, with one cage of each sex in each of 6 boxes on 12h light: 12h dark cycles, with onset of light staggered by 4h. Mice were immunized intragastrically with 2 mg/kg CEA at 2, 6, 10, 14, 18, or 22h after onset of light. Injections were repeated at 14, 27 and 35d. Mice were weighed and sacrificed approximately 40 minutes after the last injection. Relative intestine weight (RIW), an indicator of localized Type I hypersensitivity, was determined. RIW from immunized animals was different between males and females (1.056 and 1.293, respectively, $p = 0.0001$). Female RIW was significantly greater than unimmunized controls, male was not ($p = 0.03$ and > 0.05, respectively). Absolute and relative intestine weights exhibited significant circadian rhythm for experimental males and females in contrast to controls which did not display rhythms. Furthermore, IgG, A, and M specific for CEA was produced in a significant circadian rhythm by females but not by males ($p = 0.032$ vs > 0.05). Peak response to CEA was noted 14 - 18h after onset of light (early in the dark/activity span). These data suggest that female may be the preferable sex to use for a BALB/c model of food allergy.

1775 ASSESSMENT OF EXPOSURE TO 60 HZ MAGNETIC FIELDS FOR POTENTIAL IMMUNOMODULATORY EFFECTS

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Immunosuppression has been proposed as a mechanism that may underlie the reported increases in risk of leukemia and other malignancies in individuals exposed to magnetic fields. In the present studies, a modification of the National Toxicology Program (NTP) immunotoxicology Tier I bioassay was used to investigate the hypothesis that subchronic exposure to 60 Hz magnetic fields alters immune responses in B6C3F1 mice. Endpoints evaluated included, B-cell function assessed by antibody plaque forming cell (AFC) response, T-cell function assessed by delayed-type hypersensitivity response, natural killer (NK) cell function, lymphocyte subset analysis, and host resistance as assessed as survival following infectious challenge with *Listeria monocytogenes*. Except for AFC response and *Listeria* challenge, all assays were performed following 28 and 90 days of exposure; AFC response was assessed after 21 and 90 days exposure, and susceptibility to *Listeria* was assessed after 28 days exposure. Mice were exposed continuously to linearly polarized, pure sinusoidal 60 Hz fields of 10 G, 2 G, 20 mG, or 0 G, or intermittently to 10 G fields. Exposure to magnetic fields had no effect on lymphoid organ weights, cellularity, or cell viability, B-cell function, T-cell function, or host resistance to infectious challenge. Although some variability in NK response was seen, no repeatable pattern of magnetic field alterations in NK cell cytolytic activity was observed. In summary, subchronic exposure to linearly polarized 60 Hz magnetic fields appears to have no consistent adverse effect on immune parameters associated with host defense. (Supported by contract NO1-ES-25351 from the NTP/NIEHS).

1776 THE INFLUENCE OF VEHICLE GAVAGE ON SEASONALITY OF IMMUNE SYSTEM PARAMETERS IN THE B6C3F1 MOUSE

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Seasonal hyporesponsiveness and other immune system variations were observed in female B6C3F1 mice during immunomodulation screening tests. For a 5-year period, the data from over 1200 naive, vehicle, and immunosuppressant (cyclophosphamide-treated, CY) control mice were compiled and analyzed for consistency of circannual rhythm. Endpoints included body, spleen (SP), and thymus (THY) weights (both absolute and body weight-relative, %), and an immunotoxicity assessment measuring antibody formation to sheep red blood cell injection (plaque-forming cells, PFC). Dosing vehicles used were water, corn oil, or methylcellulose instilled by oral gavage in a 5-20 ml/kg volume for 5 days. Four days later, terminal organ and body weights were recorded and spleen cell antibody formation assessed by PFC assay. Circannual rhythms in naive mice occurred in a consistent pattern, with the yearly peak response (acrophase, ϕ) for body weight and PFC parameters occurring in the summer, and SP, SP%, THY and THY% in the spring. When compared to naive mice, vehicle gavage altered the ϕ in all measured endpoints, with corn oil eliminating the rhythm for PFC, SP, SP%, and THY. As expected, CY caused significant decreases ($p \leq 0.05$) in circannual mean values for PFC and organ weights when compared to naive controls, as well as eliminating expression of rhythm for THY and PFC parameters. Each vehicle caused significant reduction in circannual means for SP and SP%, with corn oil and methylcellulose also reducing THY and PFC values. These results indicate that dosing vehicle effects should be considered in toxicity evaluations, as vehicles may alter normal seasonal patterns in the mouse.

1777 RISK ASSESSMENT OF UVB EFFECTS ON RESISTANCE TO INFECTIOUS DISEASES

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We have made a first attempt to estimate the risk of UVB exposure with respect to resistance against infectious diseases. In general, in risk assessment uncertainty factors for inter- and intraspecies differences are applied for extrapolation of animal data. Using a worst case strategy the interspecies factor for UVB susceptibility is 3–4, as calculated using data from human and rat studies in which alloantigen responses and natural killer cell function were studied. Using a rat Listeria infection model UV dose response studies were performed and the dose which induced 50% suppression of the specific T cell response to Listeria was determined (ED50). This dose also impaired the clearance of Listeria. Taken this ED50 value and the uncertainty factors into account an ED50 value for suppression of cellular immunity to Listeda in man was estimated to be 13.1 kJ/m2, using exposure to UVB by FS40 lamps. In order to compare the FS40 spectrum with the solar spectrum it was necessary to calculate the biologically effective dose regarding the UVB effect on the resistance to Listeria. The wavelength action spectrum for contact hypersensitivity in mice described by De Fabo and Noonan was used to calculate this biological effective dose by multiplication of the action spectrum with the FS40 spectrum. It was calculated that the ED50 value for suppression of T cell responses to Listeria was 1.74 k J/m2. Dividing this value by the biological effective irradiance of sun exposure could give a prediction on the duration of sun exposure necessary to induce 50% suppression of specific T cell responses to Listeria in man. Using this first attempt it was calculated that 104 minutes sun exposure round noon with clear skies in July at 40°C would lead to 50% suppression of the specific T cell response to Listeria. In the case of 20% decrease of the ozone layer 94 minutes sun exposure is enough for this effect.

1778 DEVELOPMENT OF HOLISTIC CELL MEDIATED IMMUNE RESPONSE ASSAYS

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There continues to be a need to assess cell mediated immunity in a holistic manner which is congruent with a toxicology based experiment. The purpose of these studies was to develop and optimize assays to assess CD4+ and CD8+ function in mouse and rat models. Fischer 344 and Sprague Dawley rats, and B6C3F1 mice were used as responders in these experiments. DNCB and DNFB were evaluated for use as sensitizers in the CD4+ assays. Allogenic spleen cells from Brown Norway, Buffalo, Fischer 344, Long Evans, and Wistar rats and Mitomycin C treated and non-treated YAC-1 cells were evaluated for use in the CD8+ assay. In CD4+ assays, results of dose response studies to DNCB and DNFB using the ear swelling test and the local lymph node assay in mice and rats showed DNFB to give a more robust response than DNCB. For the CD8+ assay, Mitomycin C treated YAC-1 cells were chosen as the optimal cell type. Having selected the sensitizing agents, studies were conducted to demonstrate the immunosuppressive effects of cyclosporine on these assays. In rats, cyclosporine was shown to suppress the response to DNFB in the local lymph node assay by as much as 74% and the ear swelling test by up to 100%. The response to Mitomycin C treated YAC-1 cells in the CD8+ assay was shown to be suppressed by as much as 87%. These studies indicate that measuring a response to DNFB in a CD4+ assay and Mitomycin C treated YAC-1 cells in a CD8+ assay may provide good methods of assessing chemical effects on cell mediated immunity. These studies were conducted at the Medical College of Virginia Immunotoxicology Laboratory and were supported in part by E.I. duPont de Nemours and Co., Newark, DE.

1779 IMMUNOSUPPRESSIVE EFFECTS OF 2'3'-DIDEOXYINOSINE IN B6C3F1 FEMALE MICE TREATED FOR 180 DAYS

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2',3'-dideoxyinosine (ddl), a drug used in the treatment of AIDS, was investigated for selective toxicity to the immune system in B6C3F1 female mice treated twice daily for 6 months at doses between 100 and 1000 mg/kg/day. The highest dose of ddl affected body weight and body weight gain. There were minimal effects on the hematological parameters including bone marrow stem cells. No effects were seen on the liver or kidney as determined by selected serum chemistries and organ weights. The most sensitive indicator of immune toxicity was the response to the T-dependent antigen, sRBC, as measured by the IgM Antibody Forming Cell (AFC) assay and serum antibody titer. At the lowest dose, there was a 44% decrease in AFC response and 42% decrease in antibody titer; the highest dose showed an 87% and 86% decrease, respectively. Moderate effects at 1000 mg/kg were seen on the mixed leukocyte response, cytotoxic T cell response, and the reticuloendothelial ^{51}Cr sRBC clearance. The decreased response to sRBC was not believed

to be caused by a decrease in the number of T or B cells because changes in numbers were only seen at the two highest doses. Proliferation did not appear to be the reason for the defect because the cells were able to respond to both T and B cell mitogens with the exception of the T cell response at the highest dose. Thus, the action appears to be related to differentiation. Based on the data derived from these studies, the no effect level is below 100 mg/kg/day and the immune system is selectively targeted.
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1780 RAT STRAIN DIFFERENCES IN IMMUNOPATHOLOGY RESPONSE TO WHITE MINERAL OIL

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Previous 90 day feeding studies of low viscosity white oil (P15H) conducted by our laboratory have shown differences in the pathological response between F344 and Sprague Dawley derived (SD) rats. Histopathological findings in F344 rats include hepatic microgranulomas and mesenteric lymph node (MLN) histiocytosis; whereas, effects in SD rats were limited to mild inflammatory infiltrates in the liver. This study further characterizes strain differences in the nature and extent of the inflammatory cell infiltrates in liver and MLN. Frozen tissues from control and treated female F344 and SD rats fed 2% P15H were stained for T-helper and suppresser cells (CD4+, CD8+), resident macrophages (Mϕ) (ED2+), and B-cells (LCA+). Hepatic microgranulomas in treated F344 rats consisted of Mϕ infiltrates (CD4+, ED2+) surrounded by a mantle of lymphocytes with a high ratio of CD4+/CD8+; B cells were not observed. Livers of F344 control and SD test and control groups appeared normal with scattered lymphoid infiltrates, primarily CD4+. Phenotypically similar, Mϕ infiltrates were seen in the MLN of both control and test F344 groups; whereas, MLN tissues of control and test SD groups were normal. These preliminary results indicate the inflammatory response in the liver has an immune-mediated component in F344 rats. Under the conditions of this study, no evidence of immune involvement was observed in SD rats. The differential response in F344 and SD rats may be due to differences in immune susceptibility or P15H metabolism.

1781 IMMUNOHISTOCHEMICAL STUDY OF IMMUNE CELLS IN THE LIVER OF BINGE ALCOHOL TREATED RATS

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We have investigated effects of the immune response in the liver to acute binge ingestion of alcohol. Sprague-Dawley rats (n=4/group) were given one of the following treatment regimes: (a) five doses of ethanol (2g/kg) (given twice a day for 2d and then one in the AM 2hr before sacrifice) or (b) five doses (3g/kg) of ethanol or (c) five doses (3g/kg), allowed to recover 4d and then given another binge of five doses as before. Control rats were dosed in the same manner with water. Livers were surgically removed, embedded in O.C.T. compound, and snap frozen in 2-methyl butane quenched in liquid nitrogen. The frozen livers were cryosectioned and fixed in cold acetone. The tissues were immunohistochemically stained using the following monoclonal antibodies: ED2 (tissue macrophages), ED1 (monocytes, macrophages, dendritic cells), HIS48 (granulocytes), and CD54 (ICAM-1) (intracellular adhesion molecule). Periportal (PP), midzonal (MZ), and centrilobular (CL) regions of the liver were analyzed semi-quantitatively for the presence of the immune cells in treated vs control rats. Results showed that a single acute binge episode caused an increase in ED2 positive cells in PP and MZ regions, and with increased amounts of ethanol or binges there was an increase in ED2 positive cells throughout the liver. With increasing amounts of ethanol ED1 cells were increased in PP regions and HIS48 cells in PP and MZ regions. Ethanol caused increased ICAM-1 staining in the PP and an even greater increase in the CL region. ICAM-1 staining was increased in PP regions above control and an even greater increase in seen in CL regions. With the largest total dose of ethanol there is heavier staining of ICAM-1 throughout the liver compared to the other two binge treatments (reference B and C above). These studies show that acute ethanol intake has an effect on immune cell trafficking in the liver. (Supported by Center Grant P30-ES-06694, and NIAAA AA10169-02).

1782 EFFECT OF MALATHION ON MACROPHAGE FUNCTION IN MAST CELL-DEFICIENT MICE

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At noncholinergic doses, malathion was previously shown to enhance the humoral immune response and macrophage function and cause mast cell degranulation. To determine the contribution of mast cells to the alterations in macrophage function, the effects of acute administration of malathion to mast cell-deficient (MCD) mice on macrophage function was examined. Initial studies in two strains of MCD mice (6–8 weeks of age) indicated that malathion reduced macrophage function in these mice, but enhanced it in the wild type strains of mice. Reconstitution of mice of the WBB6F1-W/W^v strain with bone marrow-derived mast cells from wild type mice restored the ability of malathion to enhance macrophage function. In these studies involving reconstitution, however, malathion did not suppress immune function in the MCD mice. These mice were 16–17 weeks of age to allow age matching with the reconstitution group suggesting that suppression of macrophage function by malathion in MCD mice is age dependent. The results from these studies show that mast cells are necessary for the increase in macrophage function observed after acute oral administration of noncholinergic doses of malathion to mice. Supported by PHS ES04337.

1783 IMPLEMENTATION OF EPA REVISED CANCER ASSESSMENT GUIDELINES: INCORPORATION OF MECHANISTIC AND PHARMACOKINETIC DATA

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The EPA Cancer Assessment Guidelines were introduced in 1986 and had a profound impact on the conduct of cancer assessments in EPA and internationally. With the advances in understanding of the carcinogenic processes, it became apparent that revisions of the guidelines were needed to promote better utilization of biological data that has been evolving. In particular, there has been great interest in using pharmacokinetics and mechanism data wherever possible to replace the default use of surface area scaling and the linearized multistage quantitation procedures. The EPA undertook an extensive evaluation of its guidelines with input from national and international scientists. At the 1994 SOT annual meeting the main scientific issues were debated in an SOT forum. That SOT critique was considered by the EPA in its revision process. The revised guidelines are now in final form and undergoing implementation. The intent of this workshop is to provide hands-on experience in implementing the revised guidelines. The revision process will be described along with a discussion as to the types of mechanistic data that are needed to exploit the guidelines. Two case studies will be presented to illustrate the use of the revised cancer risk assessment guidelines with real data. Finally, a panel of representatives from industry, State and federal agencies, and the international community will discuss the guidelines with audience participation.

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